



06-05-00

A

Comparable to Form PTO/SB/05 (12/97)

Approved for use through 09/30/00, OMB0651-0032  
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

**UTILITY PATENT APPLICATION TRANSMITTAL**  
(For new Non-provisional applications under 37 CFR 1.53(b))

Attorney Docket No.: ARD7001P0041US  
First Named Inventor: M.E. Greene *et al.*  
Express Mail Label No. EL5392311477US



Box PATENT APPLICATION  
Assistant Commissioner For Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is a new utility patent application of inventor(s): **M.E. Greene and Bruce Blumberg** and entitled: **Human Peroxisome Proliferator Activated Receptor Gamma: Compositions and Methods.**

**Application Elements:**

1. ☒ Specification containing 28 pages (preferred arrangement set forth below)
  - Descriptive Title of the Invention
  - Cross-reference to related applications (if applicable)
  - Statement regarding Federally-sponsored Research & Development (if applicable)
  - Reference to Microfiche Appendix (if applicable);
  - Background of the Invention
  - Brief Summary of the Invention
  - Brief Description of the Drawings (if filed)
  - Detailed Description
  - Claim(s)
  - Abstract of the Disclosure
2. ☒ Drawings: 1 Sheets of ☐ formal drawings ☐ informal drawings
3. ☒ Oath or Declaration
  - a. ☐ An executed declaration or oath for the utility patent application including a power of attorney,
  - b. ☐ An unexecuted declaration or oath for the utility patent application including a power of attorney;
  - c. ☒ Copy from a prior application (37 CFR 1.63(d), for continuation/divisional with No. 16 completed. [Note No. 4 below.]).
    - i. ☐ Signed statement attached deleting inventor(s) named in the prior application (see 37 CFR 1.63(d)(2) and 1.33(b).
4. ☒ **For CONTINUATION or DIVISIONAL Applications only:** The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 3c, is considered as being part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.
5. ☐ Microfiche Computer Program (Appendix)

6. ☐ Nucleotide and/or Amino Acid Sequence submission. including:  
☐ Computer readable copy,  
☐ Paper copy (identical to computer copy),  
☐ Statement verifying identity of above copies.

**Accompanying Application Parts:**

7. ☐ Assignment Papers (cover sheet, document(s) and requisite fee).
8. ☐ 37 CFR 3.73(b) Statement (where there is an assignee)  
☐ Power of Attorney
9. ☐ English Translation document (if applicable)
10. ☐ Information Disclosure Statement (IDS), including PTO-1449  
☐ Copies of IDS Citations
11. ☒ Preliminary Amendment
12. ☒ Return Postcard for PTO Mail Room Date Stamp (should be specifically itemized).
13. ☒ Small Entity Statement(s)  
☒ Statement filed in prior application, status still proper and desired.
14. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed).
15. ☐ Other \_\_\_\_\_
16. ☒ **If Continuing Application**, check appropriate box and supply the requisite information below and in a preliminary amendment:  
☐ Continuation ☒ Divisional ☐ Continuation-in-part (CIP) of prior application No. 08/134,557, filed October 8, 1993.  
Prior application information: Examiner: J. Ketter Group/Art Unit: 1636

**Fee Calculation**

The filing fee has been calculated as shown below:

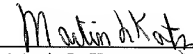
Small Entity						Large Entity	
For	No. Filed	No. Allowed	No. Extra	Rate	Fee	Rate	Fee
Basic Fee					\$345.00	OR	\$690.00
Total Claims	46	- 20 =	26	x \$9.00	\$234.00	OR	x \$18.00 \$
Indep. Claims	13	- 3 =	10	x \$39.00	\$390.00	OR	x \$78.00 \$
Multiple Dependent Claims Present				+ \$130.00	\$	OR	+ \$260.00 \$
				TOTAL	\$969.00	OR	TOTAL \$

17. ☒ A check in the amount of \$ 969.00 to cover the filing fee is enclosed.
18. ☐ Please charge my Deposit Account No. 04-1644 in the amount of \$ \_\_\_\_\_.

19. ☒ The Commissioner is authorized to charge payment of the following amounts associated with this communication or credit any overpayment to Deposit Account No. 04-1644:
- ☒ Additional filing fees under 37 CFR 1.16 or deficiencies in remittances therefor.
  - ☒ Additional processing fees under 37 CFR 1.17 or deficiencies in remittances therefor.
  - ☐ **ONLY if applicant has partially paid** the patent issue fee under 37 C.F.R. §1.18, then the **deficiency** shall be charged to Deposit Account No. 04-1644, and the Commissioner is authorized to so charge the Deposit Account.

Date: June 1, 2000

Attorney's Signature \_\_\_\_\_

  
Martin L. Katz, Reg. No. 25,011

Correspondence Address:

ROCKEY, MILNAMOW & KATZ, LTD.

Two Prudential Plaza

180 North Stetson Avenue, Suite 4700

Chicago, Illinois 60601

Telephone: (312) 616-5400

Facsimile: (312) 616-5460

**CERTIFICATE OF MAILING BY EXPRESS MAIL**

I hereby certify that this Utility Patent Application Transmittal, enclosed application, and any other documents referred to as enclosed herein, are being deposited in an envelope with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated below and addressed to Box PATENT APPLICATION, Assistant Commissioner for Patents, Washington, D.C. 20231.

Express Mail Label No.: EL5392311477US

Date of Deposit: June 1, 2000

Typed/Printed Name of Person Signing: Daniel Madrigal

Signature: 

PATENT

Applicants: Marianne E. Greene & Bruce Blumberg  
Serial No.: 08/134,557  
Docket No.:  
Filed or Issued: October 8, 1993  
For: *HUMAN PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR  
GAMMA: COMPOSITIONS AND METHODS*

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
STATUS (37CFR 1.9(f) and 1.27(d)) - NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: ARCH Development Corp.  
ADDRESS OF ORGANIZATION: 1101 E. 58th Street, Walker 213  
Chicago, IL 60637

TYPE OF ORGANIZATION:

- ☐ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION  
☒ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3))  
☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA  
(NAME OF STATE: \_\_\_\_\_)  
(CITATION OF STATUTE: \_\_\_\_\_)  
☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA  
☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA  
(NAME OF STATE: \_\_\_\_\_)  
(CITATION OF STATUTE: \_\_\_\_\_)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled: **Human Peroxisome Proliferator Activated Receptor Gamma: Compositions and Methods by Marianne Greene and Bruce Blumberg described in**

- ☐ the specification filed herewith  
☒ application serial no.: \_\_\_\_\_,  
filed:  
☐ patent no.: \_\_\_\_\_, issued: \_\_\_\_\_

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

**\*NOTE:** Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME: ARCH Development Corp.

ADDRESS: 1101 E. 58th Street, Walker 213

Chicago, IL 60637

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☒ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: STEVEN LAZARUS

TITLE IN ORGANIZATION: PRESIDENT & CEO

ADDRESS OF PERSON SIGNING: 1101 E. 58th Street

CHICAGO, IL 60637

SIGNATURE:  DATE: 3/21/94

**IN THE UNITED STATE PATENT AND TRADEMARK OFFICE**

Applicant:	M.E. Greene <i>et al.</i>	)	Attorney Docket No. ARD7001P0041US
		)	UCHI:461
Serial No.:	08/134,557	)	
		)	
Filing Date:	October 8, 1993	)	Group Art Unit: 1636
		)	
For:	Human Peroxisome Proliferator	)	Examiner: J. Ketter
	Activated Receptor Gamma:	)	
	Compositions and Methods	)	

Assistant Commissioner for Patents  
Washington, D.C. 20231

**PRELIMINARY AMENDMENT**

Sir:

In connection with filing of a Divisional Application, please amend the above-identified application as follows:

**IN THE SPECIFICATION**

Please insert on page 2, before line 1.

**Related Application**

This application is a divisional application of U.S. Serial No. 08/134,577, filed October 8, 1993.

**IN THE CLAIMS**

Please cancel claims 1-8, 12-15 and 24.

Please add the following new claims:

26. A process of screening a substance for its ability to interact with a human peroxisome proliferator activated receptor gamma polypeptide, the process comprising the steps of:

- a) providing a human peroxisome proliferator activated receptor gamma polypeptide; and

b) testing the ability of the substance to interact with the human peroxisome proliferator activated receptor gamma polypeptide.

27. The process of claim 26 wherein the substance is an agonist.

28. The process of claim 26 wherein the substance is an antagonist.

29. The process of claim 26 wherein the substance has mixed agonist and antagonist properties.

30. The process of claim 26 wherein the substance affects the transcription regulation activity of the human peroxisome proliferator activated receptor gamma polypeptide.

31. The process of claim 26 wherein the human peroxisome proliferator activated receptor gamma polypeptide is coupled to a solid support.

32. The process of claim 31 wherein the solid support is selected from the group consisting of agarose beads, polyacrylamide beads and polyacrylic beads.

33. The process of claim 26 wherein the interaction between the substance and the human peroxisome proliferator activated receptor gamma polypeptide is detected by centrifugation, chromatography, electrophoresis or spectroscopy.

34. The process of claim 26 wherein the interaction between the substance and the human peroxisome proliferator activated receptor gamma polypeptide is detected by a reporter gene.

35. The process of claim 34 wherein the reporter gene is selected from the group consisting of  $\beta$ -galactosidase, chloramphenicol acetyl transferase and luciferase.

36. The process of claim 26 wherein the substance binds to the human peroxisome proliferator activated receptor gamma polypeptide.

37. A process of screening a substance for its ability to interact with a human peroxisome proliferator activated receptor gamma polypeptide, the process comprising the steps of:

- a) providing a host cell transformed with a polynucleotide, which upon expression, encodes a human peroxisome proliferator activated receptor gamma polypeptide.
- b) maintaining the transformed host cell under biological conditions sufficient to allow for the expression of human peroxisome proliferator activated receptor gamma polypeptide; and
- c) testing the ability of the substance to interact with the human peroxisome proliferator activated receptor gamma polypeptide.

38. The process of claim 37 wherein the substance is an agonist.

39. The process of claim 37 wherein the substance is an antagonist.

40. The process of claim 37 wherein the substance has mixed agonist and antagonist properties.

41. The process of claim 37 wherein the substance affects the transcription regulation activity of the human peroxisome proliferator activated receptor gamma polypeptide.

42. The process of claim 37 wherein the host cell is a prokaryotic or eukaryotic cell.

43. The process of claim 37 further comprising the step of isolating the human peroxisome proliferator activated receptor gamma polypeptide from the host cell prior to testing the ability of the substance to interact with the human peroxisome proliferator activated receptor gamma polypeptide.

44. The process of claim 43 wherein the human peroxisome proliferator activated receptor gamma polypeptide is coupled to a solid support.



45. The process of claim 44 wherein the solid support is selected from the group consisting of agarose beads, polyacrylamide beads and polyacrylic beads.

46. The process of claim 44 wherein the interaction between the substance and the human peroxisome proliferator activated receptor gamma polypeptide is detected by centrifugation, chromatography, electrophoresis or spectroscopy.

47. The process of claim 44 wherein the interaction between the substance and the human peroxisome proliferator activated receptor gamma polypeptide is detected by a reporter gene.

48. The process of claim 44 wherein the reporter gene is selected from the group consisting of  $\beta$ -galactosidase, chloramphenicol, acetyl transferase and luciferase.

49. The process of claim 44 wherein the substance binds to the human peroxisome proliferator activated receptor gamma polypeptide.

50. A process of screening an substance for its ability to modify the function of the human peroxisome proliferator activated receptor gamma polypeptide, the process comprising the steps of:

- a) providing a human peroxisome proliferator activated receptor gamma polypeptide; and
- b) testing the ability of the substance to modify the function of the human peroxisome proliferator activated receptor gamma polypeptide.

51. The process of claim 50 wherein the substance is an agonist.

52. The process of claim 50 wherein the substance is an antagonist.

53. The process of claim 50 wherein the substance has mixed agonist and antagonist properties.

54. The process of claim 50 wherein the substance modifies the transcription regulation activity of the human peroxisome proliferator activated receptor gamma polypeptide.

55. The process of claim 50 wherein the human peroxisome proliferator activated receptor gamma polypeptide is coupled to a solid support.

56. The process of claim 55 wherein the solid support is selected from the group consisting of agarose beads, polyacrylamide beads and polyacrylic beads.

57. A process of detecting a messenger RNA transcript from a biological sample which encodes a human peroxisome proliferator activated receptor gamma polypeptide, the process comprising the steps of:

- a) hybridizing a messenger RNA transcript from a biological sample with a polynucleotide sequence to form a duplex, wherein said polynucleotide sequence, upon expression, encodes for human peroxisome proliferator activated receptor gamma polypeptide; and
- b) detecting the duplex.

58. A process of detecting DNA molecule in a biological sample, which upon expression, encodes a human peroxisome proliferator activated receptor gamma polypeptide, the process comprising the steps of:

- a) hybridizing a DNA molecule from a biological sample with a polynucleotide sequence to form a duplex, wherein said polynucleotide sequence, upon expression, encodes for human peroxisome proliferator activated receptor gamma polypeptide; and
- b) detecting the duplex.

**REMARKS**

Submitted herewith are new claims 26-58 that are directed to difference embodiments of the present invention. Support for these claims is found on pages 2, 12, 14 and 54-60.

Favorable consideration and allowance of claims 9-11, 16-23 and 25-58 is respectfully requested.

**Conclusion**

If any fees incurred as a result of the filing of this paper, authorization is given to charge Deposit Account Number 04-1644.

Respectfully submitted,

By Martin L. Katz  
Martin L. Katz, Reg. No. 25, 011

ROCKEY, MILNAMOW & KATZ, LTD.  
Two Prudential Plaza  
180 North Stetson Avenue  
Suite 4700  
Chicago, Illinois 60601  
(312) 616-5400

**CERTIFICATE OF EXPRESS MAILING**

I hereby certify that this Preliminary Amendment and any other documents referred to as enclosed herein, are being deposited in an envelope with sufficient postage with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated below and addressed to BOX PATENT APPLICATION, Assistant Commissioner for Patents, Washington, D.C. 20231.

EL539211477US  
Express Mail Label No.

Daniel Madrigal  
Typed/Printed Name of Person  
Mailing Correspondence

June 1, 2000  
Date of Deposit  
Daniel Madrigal  
Signature of Person Mailing  
Correspondence

S P E C I F I C A T I O N

(Case No. ARCD:098)

Be it known that we, **Marianne E. Greene and  
Bruce Blumberg** have invented a new and useful HUMAN  
PEROXISOME PROLIFERATOR ACTIVATED  
RECEPTOR GAMMA: COMPOSITIONS AND  
METHODS, of which the following is a specification.

**"EXPRESS MAIL" MAILING LABEL**

**Number:** TB419202135

**Date of Deposit:** 8 October 1993

*I hereby certify that this paper or fee is  
being deposited with the United States Postal  
Service as "Express Mail Post Office To  
Addressee" Service under 37 C.F.R. §1.10 on  
the date indicated above and is addressed to the  
Honorable Commissioner of Patents and  
Trademarks, Washington, D.C. 20231*

signed

*Joseph Deming*

Field of the Invention.

This invention relates generally to compositions of and methods for obtaining peroxisome proliferator-activated receptor- $\gamma$  polypeptides. The invention relates as well to the DNA sequences encoding these receptor polypeptides, the recombinant vectors carrying those sequences, the recombinant host cells including either the sequences or vectors, and recombinant peroxisome proliferator-activated receptor- $\gamma$  polypeptides. The invention includes, as well, methods for using the isolated, recombinant receptor polypeptides in assays designed to select and improve among candidate substances such as agonists and antagonists of peroxisome proliferator-activated receptor- $\gamma$  polypeptides for use in diagnostic, drug design and therapeutic applications.

Background of the Invention.

The steroid hormone (or nuclear) receptor superfamily has many members that play important roles in regulating cancer cells, including, but not limited to, the estrogen receptor (Bettuzzi, 1991), the retinoic acid receptors (Menger, 1988), and v-erb A (Sharif, 1991). A number of less well characterized members of this family have been isolated and identified based on their homology to the better known nuclear receptors. Many show ligand-dependent activation patterns, but the identity of natural ligands that control these transcription factors is still unknown, or under investigation. Members of the steroid hormone receptor family whose ligands have not yet been identified are referred to as "orphan" receptors. The full importance of these "orphan " receptors is predicted by their presence early in development and by the fact that many (including PPAR, COUP, ROR) have been shown to interact with the known members of the superfamily (Beck, 1992; O'Malley, 1992; Tsukiyama, 1992). Many of these receptors are undoubtedly important in regulating cell growth and response to the environment, and may well

have regulatory roles in cancer cells, like their currently well-studied counterparts.

The present invention provides a novel "orphan" receptor polypeptide, the human Peroxisome Proliferator Receptor  $\gamma$  (hPPAR- $\gamma$ ). It is a member of a group of orphan receptors found to be activated (but not bound) by arachidonic acid, fatty acids, clofibrate, and other agents that induce the proliferation of peroxisomes in rodents. (Blaauboer, 1990).

Peroxisome proliferators are a diverse group of chemicals which include hypolipidemic drugs, herbicides, leukotriene antagonists, and plasticizers. Two major categories of peroxisome proliferator chemicals play a significant role in society today. The first, the fibrate class of hypolipidemic drugs, has been found to be effective at reducing the levels of triglycerides and cholesterol in humans suffering from hyperlipidemia, a major risk factor for heart disease (Beroli, 1990). The second category relates to phthalate ester plasticizers used in the production of highly versatile flexible vinyl plastics (Reddy, 1983).

Peroxisome proliferators seem to affect most mammalian species that have been tested. They induce hepatomegaly resulting from liver hyperplasia and an increase in the size and number of peroxisomes. (Reddy, 1983.) Nevertheless, on the basis of hypolipidemic drug dose required to produce recognizable peroxisome proliferation, mice and rats are considered to be highly responsive to these agents, developing hepatocellular carcinoma following long-term drug administration; hamsters have intermediate responses; and guinea pigs, marmosets and other nonhuman primates are weakly responsive (Eacho, 1986).

Peroxisome proliferators are termed non-genotoxic carcinogens as they fail to cause DNA damage directly (Warren, 1980). The increase in peroxisomal fatty acid  $\beta$ -oxidation seen in response to peroxisome proliferators results in a greater production of hydrogen peroxide. It has been proposed that this results in oxidative stress leading to DNA damage and possible tumor initiation. (Reddy, 1983; Kasai, 1989). Alternatively, or in addition, peroxisome proliferators may act as liver-tumor promoters. (Marsman, 1988; Cattley, 1989).

The nuclear receptor subfamily to which the receptor polypeptide of the present invention belongs has been shown to regulate the transcription of several key enzymes in fatty acid metabolism, including Acyl Co A oxidase (Tugwood, 1992), and CYP4A6, a cytochrome P-450 omega hydroxylase, potentially having profound effects upon host responses via leukotriene and prostaglandin catabolism, fatty acid  $\beta$  oxidation, and superoxide production. Host response is a key aspect to the pathology of all diseases, including cancer, infection, and autoimmune disorders.

Recent studies demonstrate the PPARs can heterodimerize with the 9-cis retinoic acid receptor (RXR) and synergistically induce gene expression (Kliwer, 1992). These studies demonstrate a potential link between the retinoid responsive pathways and the lipid/arachidonic acid metabolic pathways. Preservation of the hydrophobic heptad repeats in the dimerization domain (see discussion below) indicates that PPARs can heterodimerize with other members of the thyroid/retinoid subbranch of the superfamily containing these repeats. There is also some conservation of the "conserved activating motif" (Daneilian *et al.*, 1992) in the carboxy terminus.

PPARs are orphan receptors described in the murine (Isseman,  
1990), rat (Gottlicher, 1992), human (Sher, 1993) and xenopus systems  
(Dreyer, 1992) as the peroxisome proliferator activated receptors  $\alpha$ ,  $\beta$ ,  
and  $\gamma$ . A fourth member (h NUC 1) with some interesting unique  
5 sequence characteristics was isolated from human osteosarcoma cells  
(Schmidt, 1992). These receptors in other species are known to be  
activated by arachidonic acid at 150  $\mu$ M levels, long chain fatty acids  
such as oleic and petroselenic acid, clofibrate, and other peroxisome  
proliferating agents (Gottlicher, 1992; Isseman, 1990). Arachidonic acid  
10 can trigger many different responses in cells such as neutrophils, but  
PPAR activation by long chain fatty acids provides evidence that  
arachidonic acid metabolites are candidate ligands or activators. In  
addition, murine PPAR- $\alpha$  has been shown to transactivate as a  
heterodimer with the human Retinoid X Receptor alpha (hRXR- $\alpha$ ) both on  
15 retinoid receptor targets as well as on known PPAR target promoter  
sequences, potentially linking these pathways of gene regulation  
(Kliewer, 1992).

PPARs may play a role in proliferative and differentiation aspects  
of cancer, because they have also been shown to be developmentally  
20 active in vertebrates (xenopus), and are present in oocytes, fertilized eggs,  
blastulae, gastrulae, neurulae, and early tadpoles (Dreyer, 1992). It  
should be noted that many other members of the nuclear receptor  
superfamily appear to have two sets of regulatory functions. The first  
occurs during embryogenesis and development, including axis and pattern  
25 formation (Blumberg, 1992), and the second occurs in the adult, where  
the receptors modulate transcriptional activities in response to neighboring  
cells, other tissues, and aspects of the environment, particularly on  
regenerating tissues (Elder, 1991; Howell, 1990).



Members of the superfamily of nuclear hormone receptors have also been shown to directly connect the cellular transcriptional response to extracellular signals such as retinoids and steroids, as well as fatty acid and arachidonic acid metabolites, as already mentioned (Aronica, 1991).  
5 Studies of these receptors in hematopoiesis have primarily focused upon the retinoic acid receptor alpha (RAR- $\alpha$ ) and v-erb A genes because of their important effects on hematopoietic cell differentiation.

The retinoic acid receptor  $\alpha$  gene on chromosome 17 is translocated to chromosome 15 in virtually all cases of human acute promyelocytic leukemia (APL) (Rowley, 1988), where it is fused with  
10 PML gene. This generates two fusion proteins, containing RAR- $\alpha$  and PML sequences, which have different transcriptional activating properties than the wild type proteins. Treatment of APL patients with the ligand, all trans retinoic acid, consistently results in the achievement of a  
15 complete remission in this disease (Menger, 1988).

V-erb A is an aberrant version of a thyroid hormone receptor that can block erythroid differentiation and induce malignant transformation, an ability that is correlated with the repression of retinoic acid receptor function. Both retinoic acid receptors and v-erb A elicit significant  
20 effects upon the hematopoietic system by interacting with other transcripti on factors, such as *fos* and *jun* (Schule, 1991), and with other related nuclear receptors leading to heterodimer formation (Debois, 1991). The retinoic acid receptors, (RARs) can heterodimerize with retinoid  $x$  receptors (RXRs) *in vitro*, which increases their affinity for the RAR  
25 response element. RXRs can also heterodimerize with a number of different members of the superfamily *in vitro*, including the PPARs (Kliewer, 1992).

Target genes identified as transcriptionally activated by PPARs include acyl co-A oxidase, the key enzyme regulating the fatty acid B oxidation pathway (Tugwell, 1992), and CYP4A6, a cytochrome P450 fatty acid  $\omega$  hydroxylase, a key enzyme catalyzing the  $\omega$  hydroxylation of arachidonic, lauric and palmitic acids (Muerhoff, 1992). The sequence in the rat acyl-Co A oxidase promoter bound by PPARs is "acgTGACCTrTGTCCTggt" (SEQ ID NO:6) (Tugwell, 1992), which contains a direct repeat, separated by one nucleotide, ("DR-1" motif) (Umesono, 1991) of the canonical consensus half site "TGACCT" (SEQ ID NO:7) to which all members of the thyroid-retinoid branch of the nuclear receptor superfamily can bind (Laudet, 1992). The target sequences in CYP4A6 appear as imperfect DR-1 motifs and more complex arrangements of imperfect half sites. Candidate gene promoters must be tested for PPAR activation, but genes such as CD18, the leukocyte integrin  $\beta$  subunit, which has multiple combinations of imperfect half-sites and is retinoic acid inducible (Agura, 1992), are possibly PPAR responsive. Catalase, hydroxyacid oxidase, and uricase are down regulated by peroxisome proliferators in the rat, under the same conditions that up regulate acyl-coA oxidase. The effects of PPAR upon myeloperoxidase, chloroacetate esterase or catalase in myeloid cells have not been examined. Further characterization of these two forms of the PPAR- $\gamma$  mRNA will be extremely useful for understanding their functions in hematopoietic cells as well as other organ systems.

#### Summary of the Invention.

In one aspect, the present invention provides an isolated and purified polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide. In a preferred embodiment, a polynucleotide of the present invention is a DNA molecule. More preferably, a polynucleotide of the present invention encodes a polypeptide that is a peroxisome

proliferator-activated receptor- $\gamma$ . Even more preferred, a polynucleotide of the present invention encodes a polypeptide comprising the amino acid residue sequence of SEQ ID NO:2. Most preferably, an isolated and purified polynucleotide of the invention comprises the nucleotide base sequence of SEQ ID NO:1.

In an alternative aspect, the present invention contemplates an isolated and purified polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide, said polynucleotide preparable by a process comprising the steps of (a) constructing a library of cDNA clones from a cell that expresses said polypeptide; (b) screening the library with a radio-labelled oligonucleotide probe; (c) identifying a clone that hybridizes to the probe; and (d) isolating the hybridized clone from the library of unhybridized clones. Preferably, the present invention provides an isolated and purified polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide prepared by a process comprising the steps of (a) constructing a library of cDNA clones from a cell that expresses said polypeptide; (b) screening the library with a radio-labelled oligonucleotide probe; (c) identifying a clone that hybridizes to the probe; and (d) isolating the hybridized clone from the library of unhybridized clones. More preferably, the polypeptide encoded has the amino acid residue sequence of SEQ ID NO: 2. Still more preferably, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 1.

Yet another aspect of the present invention contemplates an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 1, wherein the polynucleotide hybridizes to a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide.

Preferably, the isolated and purified polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of SEQ ID NO: 1. For example, the polynucleotide of the present invention can comprise a segment of nucleotide bases identical or complementary to 40 or 55 contiguous bases of the disclosed nucleotide sequences.

In still another embodiment of the present invention, there is provided an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 1. The polynucleotide of the invention hybridizes to SEQ ID NO: 1, or a complement of SEQ ID NO: 1. Preferably, the isolated and purified polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of SEQ ID NO: 1. For example, the polynucleotide of the invention can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of SEQ ID NO: 1.

In another embodiment, the present invention contemplates an isolated and purified peroxisome proliferator-activated receptor polypeptide. Preferably, a peroxisome proliferator-activated receptor polypeptide of the invention is a recombinant polypeptide. More preferably, a peroxisome proliferator-activated receptor polypeptide of the present invention is a peroxisome proliferator-activated receptor- $\gamma$  polypeptide. Even more preferably, a peroxisome proliferator-activated receptor polypeptide of the present invention comprises the amino acid residue sequence of SEQ ID NO:2.

In an alternative embodiment, the present invention provides an expression vector comprising a polynucleotide that encodes a peroxisome

proliferator-activated receptor polypeptide. Preferably, an expression vector of the present invention comprises a polynucleotide that encodes a polypeptide comprising the amino acid residue sequence of SEQ ID NO:2. More preferably, an expression vector of the present invention  
5 comprises a polynucleotide comprising the nucleotide base sequence of SEQ ID NO:1. Even more preferably, an expression vector of the invention comprises a polynucleotide operatively linked to an enhancer-promoter. More preferably still, an expression vector of the invention comprises a polynucleotide operatively linked to a prokaryotic promoter.  
10 Alternatively, an expression vector of the present invention comprises a polynucleotide operatively linked to an enhancer-promoter that is a eukaryotic promoter, and the expression vector further comprises a polyadenylation signal that is positioned 3' of the carboxy-terminal amino acid and within a transcriptional unit of the encoded polypeptide.

15 In yet another embodiment, the present invention provides a recombinant host cell transfected with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide. Preferably, a recombinant host cell of the present invention is transfected with the polynucleotide of SEQ ID NO:1. Even more preferably, a host cell of  
20 the invention is a eukaryotic host cell. Still more preferably, a recombinant host cell of the present invention is a yeast cell. Alternatively, a recombinant host cell of the invention is a COS-1 cell. In another aspect, a recombinant host cell of the present invention is a prokaryotic host cell.

25 In yet another embodiment, the present invention contemplates a process of preparing a peroxisome proliferator-activated receptor polypeptide comprising transfecting a cell with polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide to

produce a transformed host cell; and maintaining the transformed host cell under biological conditions sufficient for expression of the polypeptide. Preferably, the transformed host cell is a eukaryotic cell. More preferably still, the eukaryotic cell is a COS-I cell. Alternatively, the host cell is a prokaryotic cell. More preferably, the prokaryotic cell is a bacterial cell of the DH5 $\alpha$  strain of *Escherichia coli*. Even more preferably, a polynucleotide transfected into the transformed cell comprises the nucleotide base sequence of SEQ ID NO:1.

In still another embodiment, the present invention provides an antibody immunoreactive with a peroxisome proliferator-activated receptor polypeptide. Preferably, an antibody of the invention is a monoclonal antibody. More preferably, the antibody is immunoreactive with a peroxisome proliferator-activated receptor polypeptide that comprises the amino acid residue sequence of SEQ ID NO:2.

In another aspect, the present invention contemplates a process of producing an antibody immunoreactive with a peroxisome proliferator-activated receptor polypeptide comprising the steps of (a) transfecting a recombinant host cell with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide; (b) culturing the host cell under conditions sufficient for expression of the polypeptide; (c) recovering the polypeptide; and (d) preparing the antibody to the polypeptide. Preferably, the host cell is transfected with the polynucleotide of SEQ ID NO:1. Even more preferably, the present invention provides an antibody prepared according to the process described above.

Alternatively, the present invention provides a process of detecting a peroxisome proliferator-activated receptor polypeptide, wherein the

process comprises immunoreacting the polypeptide with an antibody prepared according to the process described above to form an antibody-polypeptide conjugate, and detecting the conjugate.

In yet another embodiment, the present invention contemplates a process of detecting a messenger RNA transcript that encodes a peroxisome proliferator-activated receptor polypeptide, wherein the process comprises (a) hybridizing the messenger RNA transcript with a polynucleotide sequence that encodes the peroxisome proliferator-activated receptor polypeptide to form a duplex; and (b) detecting the duplex.

Alternatively, the present invention provides a process of detecting a DNA molecule that encodes a peroxisome proliferator-activated receptor polypeptide, wherein the process comprises (a) hybridizing DNA molecules with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide to form a duplex; and (b) detecting the duplex.

Alternatively, the present invention contemplates a pharmaceutical composition comprising a peroxisome proliferator-activated receptor polypeptide and a physiologically acceptable carrier. Preferably, the present invention provides a pharmaceutical composition comprising a receptor polypeptide that comprises the amino acid residue sequence of SEQ ID NO:2. In another embodiment, the present invention provides a pharmaceutical composition comprising a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide, and a physiologically acceptable carrier. Preferably, that pharmaceutical composition comprises a polynucleotide encoding a receptor polypeptide comprising the amino acid residue sequence of SEQ ID NO:2. Even more preferably, the pharmaceutical composition comprises a polynucleotide that comprises the nucleotide sequence of SEQ ID NO:1.

In another aspect, the present invention contemplates a diagnostic assay kit for detecting the presence of a peroxisome proliferator-activated receptor polypeptide in a biological sample, where the kit comprises a first container containing a first antibody capable of immunoreacting with a peroxisome proliferator-activated receptor polypeptide, with the first antibody present in an amount sufficient to perform at least one assay. Preferably, an assay kit of the invention further comprises a second container containing a second antibody that immunoreacts with the first antibody. More preferably, the antibodies used in an assay kit of the present invention are monoclonal antibodies. Even more preferably, the first antibody is affixed to a solid support. More preferably still, the first and second antibodies comprise an indicator, and, preferably, the indicator is a radioactive label or an enzyme.

In an alternative aspect, the present invention provides a diagnostic assay kit for detecting the presence, in biological samples, of a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide, the kits comprising a first container that contains a second polynucleotide identical or complementary to a segment of at least 10 contiguous nucleotide bases of SEQ ID NO:1.

In another embodiment, the present invention contemplates a diagnostic assay kit for detecting the presence, in a biological sample, of an antibody immunoreactive with a peroxisome proliferator-activated receptor polypeptide, the kit comprising a first container containing a peroxisome proliferator-activated receptor polypeptide that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay.



In yet another aspect, the present invention contemplates a process of screening substances for their ability to interact with a peroxisome proliferator-activated receptor polypeptide comprising the steps of providing a peroxisome proliferator-activated receptor polypeptide, and testing the ability of selected substances to interact with the peroxisome proliferator-activated receptor polypeptide.

In a preferred embodiment, providing a peroxisome proliferator-activated receptor polypeptide is transfecting a host cell with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide to form a transformed cell and maintaining the transformed cell under biological conditions sufficient for expression of the peroxisome proliferator-activated receptor polypeptide.

#### Brief Description of the Drawing.

Figure 1: Comparative amino acid identity between hPPAR- $\gamma$ , other members of the PPAR family, and hRAR  $\alpha$ . The percentage of amino acid identity among the various receptors and hPPAR- $\gamma$  is indicated for each domain. The E domain is further subdivided to indicate the location of the highly conserved  $\tau$  1 putative silencing domain (Forman and Samuels, 1990).

#### Detailed Description of the Invention.

##### I. The Invention.

The present invention provides DNA segments, purified polypeptides, methods for obtaining antibodies, methods of cloning and using recombinant host cells necessary to obtain and use recombinant peroxisome proliferator-activated receptors. Thus, the difficulties encountered with applying the standard approaches of classical genetics or techniques in molecular biology evident in the prior art to peroxisome

proliferator-activated receptors, have been overcome. Accordingly, the present invention concerns, generally, compositions and methods for the preparation and use of peroxisome proliferator-activated receptors.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158  
159  
160  
161  
162  
163  
164  
165  
166  
167  
168  
169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339  
340  
341  
342  
343  
344  
345  
346  
347  
348  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479  
480  
481  
482  
483  
484  
485  
486  
487  
488  
489  
490  
491  
492  
493  
494  
495  
496  
497  
498  
499  
500  
501  
502  
503  
504  
505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
610  
611  
612  
613  
614  
615  
616  
617  
618  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
960  
961  
962  
963  
964  
965  
966  
967  
968  
969  
970  
971  
972  
973  
974  
975  
976  
977  
978  
979  
980  
981  
982  
983  
984  
985  
986  
987  
988  
989  
990  
991  
992  
993  
994  
995  
996  
997  
998  
999  
1000  
1001  
1002  
1003  
1004  
1005  
1006  
1007  
1008  
1009  
1010  
1011  
1012  
1013  
1014  
1015  
1016  
1017  
1018  
1019  
1020  
1021  
1022  
1023  
1024  
1025  
1026  
1027  
1028  
1029  
1030  
1031  
1032  
1033  
1034  
1035  
1036  
1037  
1038  
1039  
1040  
1041  
1042  
1043  
1044  
1045  
1046  
1047  
1048  
1049  
1050  
1051  
1052  
1053  
1054  
1055  
1056  
1057  
1058  
1059  
1060  
1061  
1062  
1063  
1064  
1065  
1066  
1067  
1068  
1069  
1070  
1071  
1072  
1073  
1074  
1075  
1076  
1077  
1078  
1079  
1080  
1081  
1082  
1083  
1084  
1085  
1086  
1087  
1088  
1089  
1090  
1091  
1092  
1093  
1094  
1095  
1096  
1097  
1098  
1099  
1100  
1101  
1102  
1103  
1104  
1105  
1106  
1107  
1108  
1109  
1110  
1111  
1112  
1113  
1114  
1115  
1116  
1117  
1118  
1119  
1120  
1121  
1122  
1123  
1124  
1125  
1126  
1127  
1128  
1129  
1130  
1131  
1132  
1133  
1134  
1135  
1136  
1137  
1138  
1139  
1140  
1141  
1142  
1143  
1144  
1145  
1146  
1147  
1148  
1149  
1150  
1151  
1152  
1153  
1154  
1155  
1156  
1157  
1158  
1159  
1160  
1161  
1162  
1163  
1164  
1165  
1166  
1167  
1168  
1169  
1170  
1171  
1172  
1173  
1174  
1175  
1176  
1177  
1178  
1179  
1180  
1181  
1182  
1183  
1184  
1185  
1186  
1187  
1188  
1189  
1190  
1191  
1192  
1193  
1194  
1195  
1196  
1197  
1198  
1199  
1200  
1201  
1202  
1203  
1204  
1205  
1206  
1207  
1208  
1209  
1210  
1211  
1212  
1213  
1214  
1215  
1216  
1217  
1218  
1219  
1220  
1221  
1222  
1223  
1224  
1225  
1226  
1227  
1228  
1229  
1230  
1231  
1232  
1233  
1234  
1235  
1236  
1237  
1238  
1239  
1240  
1241  
1242  
1243  
1244  
1245  
1246  
1247  
1248  
1249  
1250  
1251  
1252  
1253  
1254  
1255  
1256  
1257  
1258  
1259  
1260  
1261  
1262  
1263  
1264  
1265  
1266  
1267  
1268  
1269  
1270  
1271  
1272  
1273  
1274  
1275  
1276  
1277  
1278  
1279  
1280  
1281  
1282  
1283  
1284  
1285  
1286  
1287  
1288  
1289  
1290  
1291  
1292  
1293  
1294  
1295  
1296  
1297  
1298  
1299  
1300  
1301  
1302  
1303  
1304  
1305  
1306  
1307  
1308  
1309  
1310  
1311  
1312  
1313  
1314  
1315  
1316  
1317  
1318  
1319  
1320  
1321  
1322  
1323  
1324  
1325  
1326  
1327  
1328  
1329  
1330  
1331  
1332  
1333  
1334  
1335  
1336  
1337  
1338  
1339  
1340  
1341  
1342  
1343  
1344  
1345  
1346  
1347  
1348  
1349  
1350  
1351  
1352  
1353  
1354  
1355  
1356  
1357  
1358  
1359  
1360  
1361  
1362  
1363  
1364  
1365  
1366  
1367  
1368  
1369  
1370  
1371  
1372  
1373  
1374  
1375  
1376  
1377  
1378  
1379  
1380  
1381  
1382  
1383  
1384  
1385  
1386  
1387  
1388  
1389  
1390  
1391  
1392  
1393  
1394  
1395  
1396  
1397  
1398  
1399  
1400  
1401  
1402  
1403  
1404  
1405  
1406  
1407  
1408  
1409  
1410  
1411  
1412  
1413  
1414  
1415  
1416  
1417  
1418  
1419  
1420  
1421  
1422  
1423  
1424  
1425  
1426  
1427  
1428  
1429  
1430  
1431  
1432  
1433  
1434  
1435  
1436  
1437  
1438  
1439  
1440  
1441  
1442  
1443  
1444  
1445  
1446  
1447  
1448  
1449  
1450  
1451  
1452  
1453  
1454  
1455  
1456  
1457  
1458  
1459  
1460  
1461  
1462  
1463  
1464  
1465  
1466  
1467  
1468  
1469  
1470  
1471  
1472  
1473  
1474  
1475  
1476  
1477  
1478  
1479  
1480  
1481  
1482  
1483  
1484  
1485  
1486  
1487  
1488  
1489  
1490  
1491  
1492  
1493  
1494  
1495  
1496  
1497  
1498  
1499  
1500  
1501  
1502  
1503  
1504  
1505  
1506  
1507  
1508  
1509  
1510  
1511  
1512  
1513  
1514  
1515  
1516  
1517  
1518  
1519  
1520  
1521  
1522  
1523  
1524  
1525  
1526  
1527  
1528  
1529  
1530  
1531  
1532  
1533  
1534  
1535  
1536  
1537  
1538  
1539  
1540  
1541  
1542  
1543  
1544  
1545  
1546  
1547  
1548  
1549  
1550  
1551  
1552  
1553  
1554  
1555  
1556  
1557  
1558  
1559  
1560  
1561  
1562  
1563  
1564  
1565  
1566  
1567  
1568  
1569  
1570  
1571  
1572  
1573  
1574  
1575  
1576  
1577  
1578  
1579  
1580  
1581  
1582  
1583  
1584  
1585  
1586  
1587  
1588  
1589  
1590  
1591  
1592  
1593  
1594  
1595  
1596  
1597  
1598  
1599  
1600  
1601  
1602  
1603  
1604  
1605  
1606  
1607  
1608  
1609  
1610  
1611  
1612  
1613  
1614  
1615  
1616  
1617  
1618  
1619  
1620  
1621  
1622  
1623  
1624  
1625  
1626  
1627  
1628  
1629  
1630  
1631  
1632  
1633  
1634  
1635  
1636  
1637  
1638  
1639  
1640  
1641  
1642  
1643  
1644  
1645  
1646  
1647  
1648  
1649  
1650  
1651  
1652  
1653  
1654  
1655  
1656  
1657  
1658  
1659  
1660  
1661  
1662  
1663  
1664  
1665  
1666  
1667  
1668  
1669  
1670  
1671  
1672  
1673  
1674  
1675  
1676  
1677  
1678  
1679  
1680  
1681  
1682  
1683  
1684  
1685  
1686  
1687  
1688  
1689  
1690  
1691  
1692  
1693  
1694  
1695  
1696  
1697  
1698  
1699  
1700  
1701  
1702  
1703  
1704  
1705  
1706  
1707  
1708  
1709  
1710  
1711  
1712  
1713  
1714  
1715  
1716  
1717  
1718  
1719  
1720  
1721  
1722  
1723  
1724  
1725  
1726  
1727  
1728  
1729  
1730  
1731  
1732  
1733  
1734  
1735  
1736  
1737  
1738  
1739  
1740  
1741  
1742  
1743  
1744  
1745  
1746  
1747  
1748  
1749  
1750  
1751  
1752  
1753  
1754  
1755  
1756  
1757  
1758  
1759  
1760  
1761  
1762  
1763  
1764  
1765  
1766  
1767  
1768  
1769  
1770  
1771  
1772  
1773  
1774  
1775  
1776  
1777  
1778  
1779  
1780  
1781  
1782  
1783  
1784  
1785  
1786  
1787  
1788  
1789  
1790  
1791  
1792  
1793  
1794  
1795  
1796  
1797  
1798  
1799  
1800  
1801  
1802  
1803  
1804  
1805  
1806  
1807  
1808  
1809  
1810  
1811  
1812  
1813  
1814  
1815  
1816  
1817  
1818  
1819  
1820  
1821  
1822  
1823  
1824  
1825  
1826  
1827  
1828  
1829  
1830  
1831  
1832  
1833  
1834  
1835  
1836  
1837  
1838  
1839  
1840  
1841  
1842  
1843  
1844  
1845  
1846  
1847  
1848  
1849  
1850  
1851  
1852  
1853  
1854  
1855  
1856  
1857  
1858  
1859  
1860  
1861  
1862  
1863  
1864  
1865  
1866  
1867  
1868  
1869  
1870  
1871  
1872  
1873  
1874  
1875  
1876  
1877  
1878  
1879  
1880  
1881  
1882  
1883  
1884  
1885  
1886  
1887  
1888  
1889  
1890  
1891  
1892  
1893  
1894  
1895  
1896  
1897  
1898  
1899  
1900  
1901  
1902  
1903  
1904  
1905  
1906  
1907  
1908  
1909  
1910  
1911  
1912  
1913  
1914  
1915  
1916  
1917  
1918  
1919  
1920  
1921  
1922  
1923  
1924  
1925  
1926  
1927  
1928  
1929  
1930  
1931  
1932  
1933  
1934  
1935  
1936  
1937  
1938  
1939  
1940  
1941  
1942  
1943  
1944  
1945  
1946  
1947  
1948  
1949  
1950  
1951  
1952  
1953  
1954  
1955  
1956  
1957  
1958  
1959  
1960  
1961  
1962  
1963  
1964  
1965  
1966  
1967  
1968  
1969  
1970  
1971  
1972  
1973  
1974  
1975  
1976  
1977  
1978  
1979  
1980  
1981  
1982  
1983  
1984  
1985  
1986  
1987  
1988  
1989  
1990  
1991  
1992  
1993  
1994  
1995  
1996  
1997  
1998  
1999  
2000  
2001  
2002  
2003  
2004  
2005  
2006  
2007  
2008  
2009  
2010  
2011  
2012  
2013  
2014  
2015  
2016  
2017  
2018  
2019  
2020  
2021  
2022  
2023  
2024  
2025  
2026  
2027  
2028  
2029  
2030  
2031  
2032  
2033  
2034  
2035  
2036  
2037  
2038  
2039  
2040  
2041  
2042  
2043  
2044  
2045  
2046  
2047  
2048  
2049  
2050  
2051  
2052  
2053  
2054  
2055  
2056  
2057  
2058  
2059  
2060  
2061  
2062  
2063  
2064  
2065  
2066  
2067  
2068  
2069  
2070  
2071  
2072  
2073  
2074  
2075  
2076  
2077  
2078  
2079  
2080  
2081  
2082  
2083  
2084  
2085  
2086  
2087  
2088  
2089  
2090  
2091  
2092  
2093  
2094  
2095  
2096  
2097  
2098  
2099  
2100  
2101  
2102  
2103  
2104  
2105  
2106  
2107  
2108  
2109  
2110  
2111  
2112  
2113  
2114  
2115  
2116  
2117  
2118  
2119  
2120  
2121  
2122  
2123  
2124  
2125  
2126  
2127  
2128  
2129  
2130  
2131  
2132  
2133  
2134  
2135  
2136  
2137  
2138  
2139  
2140  
2141  
2142  
2143  
2144  
2145  
2146  
2147  
2148  
2149  
2150  
2151  
2152  
2153  
2154  
2155  
2156  
2157  
2158  
2159  
2160  
2161  
2162  
2163  
2164  
2165  
2166  
2167  
2168  
2169  
2170  
2171  
2172  
2173  
2174  
2175  
2176  
2177  
2178  
2179  
2180  
2181  
2182  
2183  
2184  
2185  
2186  
2187  
2188  
2189  
2190  
2191  
2192  
2193  
2194  
2195  
2196  
2197  
2198  
2199  
2200  
2201  
2202  
2203  
2204  
2205  
2206  
2207  
2208  
2209  
2210  
2211  
2212  
2213  
2214  
2215  
2216  
2217  
2218  
2219  
2220  
2221  
2222  
2223  
2224  
2225  
2226

## II. Polynucleotides.

- A. Isolated and purified polynucleotides that encode  
peroxisome proliferator-activated receptor polypeptides.

In one aspect, the present invention provides an isolated and  
5 purified polynucleotide that encodes a peroxisome proliferator-activated  
receptor polypeptide. In a preferred embodiment, a polynucleotide of the  
present invention is a DNA molecule. More preferably, a polynucleotide  
of the present invention encodes a polypeptide that is a peroxisome  
proliferator-activated receptor- $\gamma$ . Even more preferred, a polynucleotide  
10 of the present invention encodes a polypeptide comprising the amino acid  
residue sequence of SEQ ID NO:2. Most preferably, an isolated and  
purified polynucleotide of the invention comprises the nucleotide base  
sequence of SEQ ID NO:1.

As used herein, the term "polynucleotide" means a sequence of  
15 nucleotides connected by phosphodiester linkages. Polynucleotides are  
presented herein in the direction from the 5' to the 3' direction. A  
polynucleotide of the present invention can comprise from about 680 to  
about several hundred thousand base pairs. Preferably, a polynucleotide  
comprises from about 680 to about 150,000 base pairs. Preferred lengths  
20 of particular polynucleotide are set forth hereinafter.

A polynucleotide of the present invention can be a  
deoxyribonucleic acid (DNA) molecule or ribonucleic acid (RNA)  
molecule. Where a polynucleotide is a DNA molecule, that molecule can  
be a gene or a cDNA molecule. Nucleotide bases are indicated herein by  
25 a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C),  
inosine (I) and uracil (U).

A polynucleotide of the present invention can be prepared using standard techniques well known to one of skill in the art. The preparation of a cDNA molecule encoding a peroxisome proliferator-activated receptor polypeptide of the present invention is described hereinafter in Example 1. A polynucleotide can also be prepared from genomic DNA libraries using lambda phage technologies.

In an alternative aspect, the present invention contemplates an isolated and purified polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide, said polynucleotide preparable by a process comprising the steps of (a) constructing a library of cDNA clones from a cell that expresses said polypeptide; (b) screening the library with a radio-labelled oligonucleotide probe; (c) identifying a clone that hybridizes to the probe; and (d) isolating the hybridized clone from the library of unhybridized clones. Preferably, the present invention provides an isolated and purified polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide prepared by a process comprising the steps of (a) constructing a library of cDNA clones from a cell that expresses said polypeptide; (b) screening the library with a radio-labelled oligonucleotide probe; (c) identifying a clone that hybridizes to the probe; and (d) isolating the hybridized clone from the library of unhybridized clones. More preferably, the polypeptide encoded has the amino acid residue sequence of SEQ ID NO: 2. Still more preferably, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 1.

B. Probes and Primers.

In another aspect, DNA sequence information provided by the present invention allows for the preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to gene

sequences of the selected polynucleotides disclosed herein. In these aspects, nucleic acid probes of an appropriate length are prepared based on a consideration of a selected nucleotide sequence, e.g., a sequence such as that shown in SEQ ID NO: 1. The ability of such nucleic acid probes to specifically hybridize to a polynucleotide encoding a peroxisome proliferator-activated receptor lends them particular utility in a variety of embodiments. Most importantly, the probes can be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying or mutating a defined segment of a gene or polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide from mammalian cells using polymerase chain reactive (PCR) technology.

To provide certain of the advantages in accordance with the present invention, a preferred nucleic acid sequence employed for hybridization studies or assays includes probe molecules that are complementary to at least a 10 to 70 or so long nucleotide stretch of a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide, such as that shown in SEQ ID NO: 1. A size of at least 10 nucleotides in length helps to ensure that the fragment will be of sufficient length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 10 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of

25 to 40 nucleotides, 55 to 70 nucleotides, or even longer where desired. Such fragments can be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR technology of U.S. Patent  
5 4,603,102, herein incorporated by reference, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction enzyme sites.

Yet another aspect of the present invention contemplates an isolated and purified polynucleotide comprising a base sequence that is  
10 identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 1, wherein the polynucleotide hybridizes to a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide. Preferably, the isolated and purified polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to  
15 70 contiguous bases of SEQ ID NO: 1. For example, the polynucleotide of the present invention can comprise a segment of nucleotide bases identical or complementary to 40 or 55 contiguous bases of the disclosed nucleotide sequences.

In still another embodiment of the present invention, there is  
20 provided an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 1. The polynucleotide of the invention hybridizes to SEQ ID NO: 1, or a complement of SEQ ID NO: 1. Preferably, the isolated and purified polynucleotide comprises a base  
25 sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of SEQ ID NO: 1. For example, the polynucleotide of the invention can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of SEQ ID NO: 1.

Accordingly, a polynucleotide probe molecule of the invention can be used for its ability to selectively form duplex molecules with complementary stretches of the gene. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degree of selectivity of the probe toward the target sequence. For applications requiring a high degree of selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids. For example, one will select relatively low salt and/or high temperature conditions, such as provided by 0.02 M-0.15 M NaCl at temperatures of 50°C to 70°C. Those conditions are particularly selective, and tolerate little, if any, mismatch between the probe and the template or target strand.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate a peroxisome proliferator-activated receptor polypeptide coding sequence from other cells, functional equivalents, or the like, less stringent hybridization conditions are typically needed to allow formation of the heteroduplex. In these circumstances, one can desire to employ conditions such as 0.15 M-0.9 M salt, at temperatures ranging from 20°C to 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control of hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it is advantageous to employ a polynucleotide of the present invention in combination with an appropriate label for detecting hybrid formation. A wide variety of appropriate labels are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal.

In general, it is envisioned that a hybridization probe described herein is useful both as a reagent in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions depend as is well known in the art on the particular circumstances and criteria required (e.g., on the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe). Following washing of the matrix to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label.

## II. Peroxisome Proliferator-Activated Receptor Polypeptides.

In another embodiment, the present invention contemplates an isolated and purified peroxisome proliferator-activated receptor polypeptide. Preferably, a peroxisome proliferator-activated receptor polypeptide of the invention is a recombinant polypeptide. More preferably, a peroxisome proliferator-activated receptor polypeptide of the present invention is a peroxisome proliferator-activated receptor- $\gamma$  polypeptide. Even more preferably, a peroxisome proliferator-activated receptor polypeptide of the present invention comprises the amino acid residue sequence of SEQ ID NO:2.



Polypeptides are disclosed herein as amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus. In accordance with standard nomenclature, amino acid residue sequences are denominated by either a single letter or a three letter code as indicated below.

5

10

15

20

25

<u>Amino Acid Residue</u>	<u>3-Letter Code</u>	<u>1-Letter Code</u>
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Modifications and changes can be made in the structure of a polypeptide of the present invention and still obtain a molecule having like

peroxisome proliferator-activated receptor characteristics. For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of receptor activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a polypeptide with like properties.

In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art (Kyte & Doolittle, 1982). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, such as enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid can be substituted by another amino acid having a similar hydropathic index and still obtain

a functionally equivalent polypeptide. In such changes, the substitution of amino acids whose hydropathic indices are within  $\pm 2$  is preferred, those which are within  $\pm 1$  are particularly preferred, and those within  $\pm 0.5$  are even more particularly preferred.

5 Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biological functional equivalent polypeptide or peptide thereby created is intended for use in immunological embodiments. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a  
10 polypeptide, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e. with a biological property of the polypeptide.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0  $\pm$  1); glutamate (+3.0  $\pm$  1); serine (+0.3);  
15 asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5  $\pm$  1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an  
20 amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within  $\pm 2$  is preferred, those which are within  $\pm 1$  are particularly preferred, and those within  
25  $\pm 0.5$  are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents,

for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine (see table, below). The present invention thus contemplates functional or biological equivalents of a peroxisome proliferator-activated receptor polypeptide as set forth above.

<u>Original Residue</u>	<u>Exemplary Substitutions</u>
Ala	Gly; Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Ala
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg
Met	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Biological or functional equivalents of a polypeptide can also be prepared using site-specific mutagenesis. Site-specific mutagenesis is a technique useful in the preparation of second generation polypeptides, or biologically functional equivalent polypeptides or peptides, derived from the sequences thereof, through specific mutagenesis of the underlying DNA. As noted above, such changes can be desirable where amino acid substitutions are desirable. The technique further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to 25 nucleotides in length is preferred, with about 5 to 10 residues on both sides of the junction of the sequence being altered.

In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by *Adelman, et al.*, 1983. As will be appreciated, the technique typically employs a phage vector which can exist in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage (*Messing et al.* 1981). These phage are commercially available and their use is generally known to those of skill in the art.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector which includes within its sequence a DNA sequence which encodes all or a portion of the peroxisome proliferator-activated receptor polypeptide sequence selected. An oligonucleotide primer bearing the desired mutated sequence is prepared,

generally synthetically, for example, by the method of Crea, *et al.*, 1978. This primer is then annealed to the singled-stranded vector, and extended by the use of enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells such as *E. coli* cells and clones are selected which include recombinant vectors bearing the mutation. Commercially available kits come with all the reagents necessary, except the oligonucleotide primers.

A polypeptide of the present invention is prepared by standard techniques well known to those skilled in the art. Such techniques include, but are not limited to, isolation and purification from tissues known to contain that polypeptide, and expression from cloned DNA that encodes such a polypeptide using transformed cells (See Example 1, *infra*.)

#### Analysis of receptor functional regions.

A sizeable amount of information about an orphan receptor is gained from comparisons of the nucleotide sequence and primary structure to those of other steroid hormone receptors, because within the superfamily, functional regions tend to exist as structural modules. The steroid hormone receptor superfamily of transcription factors (Evans, 1988; Short, 1988) are, generally, structurally organized as follows:

NH <sub>2</sub> -	A/B	C	D	E	F	-COOH
-------------------	-----	---	---	---	---	-------

Region C contains two zinc fingers; the first finger and adjacent sequence contains the region that binds to the target DNA and the second finger is apparently involved in dimerization with other steroid hormone receptors. The A and B regions have some transcriptional activating

functions, as do regions C,E, and F. A basic region located between C and D, appears to be involved in nuclear localization of the receptor. The E region is the ligand binding domain. Steroid hormone receptors generally bind ligands that are small lipophilic molecules that can pass through cell membranes passively, (e.g. estrogen, progesterone, retinoic acid, or vitamin D.) The E region also is involved in nuclear localization and in binding heat shock protein. Region F, when present, can be involved in transcriptional activation.

Regulation of gene expression in a cell is accomplished through many different mechanisms. A well known mechanism of gene expression regulation is through the use of zinc finger motifs in a polypeptide. The zinc finger motif found in many transcription factors binds to DNA to regulate transcription. Zinc fingers have been identified in many transcription factors including Sp1, estrogen, and glucocorticoid receptors, several *Drosophila* developmental regulators, and the *Xenopus* Xf in protein, as well as in the *E. coli* UvrA protein and certain retroviral nucleic acid binding proteins.

Zinc fingers are postulated to bind to the major groove of B-DNA so as to interact with ~5 successive base pairs; that is, with about a half-turn of B-DNA. A zinc finger protein thus can bind to DNA in which the protein binds along one face of the DNA with successive zinc fingers bound in the major groove on alternate sides of the double helix. Zinc fingers likely form structural "scaffolds" that match the double helix's three dimensional contour. Base sequence specificity is presumably provided by the particular sequence of each zinc finger's variable residues (Klug and Rhodes, 1987).

In another embodiment, the polynucleotide that encodes for the zinc finger polypeptide or the zinc finger polypeptide can be used to identify other polynucleotides that encode a zinc finger polypeptide.

There are 3 PPAR genes described in xenopus, PPAR  $\alpha$ ,  $\beta$  and  $\gamma$ . (Dreyer, 1992). Human PPAR- $\alpha$  has been recently described, although putatively described as a single gene, unlike the xenopus genes, with no closely related family members (Sher, 1993). In the Xenopus system, PPAR  $\alpha$ ,  $\beta$  and  $\gamma$  show very high amino acid identity in their DNA binding and ligand binding regions, and correspondingly have shown similar abilities to activate the same target DNA sequences, and to be activated by clofibrate and peroxisome proliferator agents (Dreyer, 1992). However, human PPAR  $\alpha$ , and now  $\gamma$ , have different chromosomal locations. All species  $\alpha$ ,  $\beta$ , and  $\gamma$  show differences in A/B and D regions, and all species demonstrate that  $\alpha$ ,  $\beta$ , and  $\gamma$  have different tissue expression patterns. Amino acid identity between the xenopus  $\gamma$  and human  $\gamma$  receptors, in critical regions, is high enough to expect similar function, and indeed, studies on the human PPAR  $\alpha$  by Sher *et al.* (1993) show it has the same ability as xenopus and murine PPAR  $\alpha$  to activate the same reporters. This indicates that the receptor polypeptide of the present invention, human PPAR- $\gamma$ , is likely to activate these reporters in a manner similar to xenopus PPAR- $\gamma$ , based on the highly conserved identity in critical regions. However, differences in sequence are significant enough to expect differences *in vivo*.

The 108-110 aa A/B region [Wahli, 1991 #31] (transactivation domain) is approximately the same size as in the murine PPAR- $\alpha$  (102 aa) (Issemann, 1990) and the xenopus PPAR- $\gamma$  receptor (112 aa) (Dreyer, 1992) and shows highest identity to the xenopus PPAR- $\gamma$  receptor (57%). The highly conserved DNA binding C region (DNA binding and dimerization domain) has 97% aa identity with the xenopus PPAR- $\gamma$  receptor. The D region or linker region (nuclear localization domain), also referred to as the ligand I domain (Forman and Samuels, 1990), will generally show the least conservation between nuclear receptor superfamily members binding different ligands, but in subfamilies binding the same ligand there are areas of fairly high identity, and the PPAR family illustrates this concept. As



illustrated in FIG. 1, the D region here shows the highest degree of identity (70%) between the PPAR- $\gamma$ s of man and frog. The E region (ligand binding, dimerization, nuclear localization, transactivation domain) of xenopus and human PPAR- $\gamma$  overall had 75% identity. Closer examination of xenopus and human domains within the E region shows only 7% identity in the first 36 aa after the D region, 90% identity in the 42 aa T1 transactivation silencing subdomain (Forman and Samuels, 1990) and 91% identity in the region extending from the ligand binding and dimerization domains to the carboxyl terminus, (148 aa).

The open reading frame encodes nuclear receptor A/B, C, D, and E regions, and aligns with other members of the family. hPPAR- $\gamma$  1 shows 74% amino acid identity in the DNA binding and hinge regions with the murine PPAR  $\alpha$  protein, and 90% amino acid identity with the xenopus PPAR  $\gamma$  protein in these same regions, hence the designation as a full length human PPAR- $\gamma$ .

Of particular note is the conservation of the "CEGCKGFFRRTIRLK" (SEQ ID NO:8) motif in the C region. This corresponds to the portion of the first cysteine finger of the hPPAR- $\gamma$  protein that contacts the DNA in PPAR response elements (PPREs) of genes regulated by hPPAR- $\gamma$  (Kliwer, 1992; O'Malley, 1992; Truss *et al.*, 1993). Conservation of this motif is a strong indicator that this receptor too, will activate the PPREs shown to be regulated by other members of the family, and that it is likely to bind the classic estrogen/retinoid/thyroid receptor half site "AGGTCA" (Truss *et al.*, 1993). Whether it will prefer to bind this half site in direct repeat with another, with one nucleotide spaced between the repeats, referred to as a "DR-1" binding site (Umesono, 1991), as has been shown with murine PPAR- $\alpha$ , is likely. Xenopus PPAR- $\gamma$  did activate this type of response element (Dreyer *et al.*, 1992). Also of interest is the conservation of the ligand binding domain, region E. Beginning with the highly conserved T1

putative silencing domain, there is very high conservation through the dimerization domain, in particular, of the heptad repeats that have been implicated in heterodimerization (Forman and Samuels, 1990). It should be noted that the points of difference in the D and early E regions may be significant and alter ligand binding.

The shorter transcript observed on northern blot analyses is approximately 0.65 kb, and if the polyadenylation signal was not long, this would encode a predicted protein of approximately 26 kDa. In order for this transcript to encode even a short A/B region, complete C region, short D region and intact E region, it would require at least 1000 base pairs. Thus, the short transcript, if translated, would produce a nuclear receptor that lacks part or all of entire functional regions normally present in members of the superfamily.

Data indicates that the 0.65 kb mRNA was not the result of partial degradation of the RNA. However, mRNA of this size were present on more than one northern blot. Hybridization of these blots with other cDNAs, (c-fos, c-jun, RAR  $\alpha$ , and  $\beta$ -actin) showed no evidence for degradation of those mRNAs.

### III. Expression Vectors

In an alternative embodiment, the present invention provides an expression vector comprising a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide. Preferably, an expression vector of the present invention comprises a polynucleotide that encodes a polypeptide comprising the amino acid residue sequence of SEQ ID NO:2. More preferably, an expression vector of the present invention comprises a polynucleotide comprising the nucleotide base sequence of SEQ ID NO:1. Even more preferably, an expression vector of the invention comprises a polynucleotide operatively linked to an enhancer-promoter. More preferably

still, an expression vector of the invention comprises a polynucleotide operatively linked to a prokaryotic promoter. Alternatively, an expression vector of the present invention comprises a polynucleotide operatively linked to an enhancer-promoter that is a eukaryotic promoter, and the expression  
5 vector further comprises a polyadenylation signal that is positioned 3' of the carboxy-terminal amino acid and within a transcriptional unit of the encoded polypeptide.

A promoter is a region of a DNA molecule typically within about 100 nucleotide pairs in front of (upstream of) the point at which transcription  
10 begins (i.e., a transcription start site). That region typically contains several types of DNA sequence elements that are located in similar relative positions in different genes. As used herein, the term "promoter" includes what is referred to in the art as an upstream promoter region, a promoter region or a promoter of a generalized eukaryotic RNA Polymerase II transcription unit.

Another type of discrete transcription regulatory sequence element is an enhancer. An enhancer provides specificity of time, location and  
15 expression level for a particular encoding region (e.g., gene). A major function of an enhancer is to increase the level of transcription of a coding sequence in a cell that contains one or more transcription factors that bind to  
20 that enhancer. Unlike a promoter, an enhancer can function when located at variable distances from transcription start sites so long as a promoter is present.

As used herein, the phrase "enhancer-promoter" means a composite unit that contains both enhancer and promoter elements. An enhancer-  
25 promoter is operatively linked to a coding sequence that encodes at least one gene product. As used herein, the phrase "operatively linked" means that an enhancer-promoter is connected to a coding sequence in such a way that the transcription of that coding sequence is controlled and regulated by that

enhancer-promoter. Means for operatively linking an enhancer-promoter to a coding sequence are well known in the art. As is also well known in the art, the precise orientation and location relative to a coding sequence whose transcription is controlled, is dependent *inter alia* upon the specific nature of the enhancer-promoter. Thus, a TATA (SEQ ID NO:9) box minimal promoter is typically located from about 25 to about 30 base pairs upstream of a transcription initiation site and an upstream promoter element is typically located from about 100 to about 200 base pairs upstream of a transcription initiation site. In contrast, an enhancer can be located downstream from the initiation site and can be at a considerable distance from that site.

An enhancer-promoter used in a vector construct of the present invention can be any enhancer-promoter that drives expression in a cell to be transfected. By employing an enhancer-promoter with well-known properties, the level and pattern of gene product expression can be optimized.

A coding sequence of an expression vector is operatively linked to a transcription terminating region. RNA polymerase transcribes an encoding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA). Transcription-terminating regions are well known in the art. A preferred transcription-terminating region used in an adenovirus vector construct of the present invention comprises a polyadenylation signal of SV40 or the protamine gene.

An expression vector comprises a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide. Such a polypeptide is meant to include a sequence of nucleotide bases encoding a peroxisome proliferator-activated receptor polypeptide sufficient in length to distinguish said segment from a polynucleotide segment encoding a non-peroxisome proliferator-activated receptor polypeptide. A polypeptide of the invention can also encode biologically functional polypeptides or peptides which have variant amino acid sequences, such as with changes selected based on considerations such as the relative hydropathic score of the amino acids being exchanged. These variant sequences are those isolated from natural sources or induced in the sequences disclosed herein using a mutagenic procedure such as site-directed mutagenesis.

Preferably, the expression vectors of the present invention comprise polynucleotide that encode polypeptides comprising the amino acid residue sequence of SEQ ID NO: 2. An expression vector can include a peroxisome proliferator-activated receptor polypeptide coding region itself of any of the peroxisome proliferator-activated receptor polypeptides noted above or it can contain coding regions bearing selected alterations or modifications in the basic coding region of such a peroxisome proliferator-activated receptor polypeptide. Alternatively, such vectors or fragments can code larger polypeptides or polypeptides which nevertheless include the basic coding region. In any event, it should be appreciated that due to codon redundancy as well as biological functional equivalence, this aspect of the invention is not limited to the particular DNA molecules corresponding to the polypeptide sequences noted above.

Exemplary vectors include the mammalian expression vectors of the pCMV family including pCMV6b and pCMV6c (Chiron Corp., Emeryville CA). In certain cases, and specifically in the case of these individual mammalian expression vectors, the resulting constructs can require co-

transfection with a vector containing a selectable marker such as pSV2neo. Via co-transfection into a dihydrofolate reductase-deficient Chinese hamster ovary cell line, such as DG44, clones expressing peroxisome proliferator-activated receptor polypeptides by virtue of DNA incorporated into such expression vectors can be detected.

A DNA molecule of the present invention can be incorporated into a vector by a number of techniques which are well known in the art. For instance, the vector pUC18 has been demonstrated to be of particular value. Likewise, the related vectors M13mp18 and M13mp19 can be used in certain embodiments of the invention, in particular, in performing dideoxy sequencing.

An expression vector of the present invention is useful both as a means for preparing quantities of the peroxisome proliferator-activated receptor polypeptide-encoding DNA itself, and as a means for preparing the encoded polypeptide and peptides. It is contemplated that where peroxisome proliferator-activated receptor polypeptides of the invention are made by recombinant means, one can employ either prokaryotic or eukaryotic expression vectors as shuttle systems. However, in that prokaryotic systems are usually incapable of correctly processing precursor polypeptides and, in particular, such systems are incapable of correctly processing membrane associated eukaryotic polypeptides, and since eukaryotic peroxisome proliferator-activated receptor polypeptides are anticipated using the teaching of the disclosed invention, one likely expresses such sequences in eukaryotic hosts. However, even where the DNA segment encodes a eukaryotic peroxisome proliferator-activated receptor polypeptide, it is contemplated that prokaryotic expression can have some additional applicability. Therefore, the invention can be used in combination with vectors which can shuttle between the eukaryotic and prokaryotic cells. Such a system is

described herein which allows the use of bacterial host cells as well as eukaryotic host cells.

Where expression of recombinant peroxisome proliferator-activated receptor polypeptides is desired and a eukaryotic host is contemplated, it is most desirable to employ a vector such as a plasmid, that incorporates a eukaryotic origin of replication. Additionally, for the purposes of expression in eukaryotic systems, one desires to position the peroxisome proliferator-activated receptor encoding sequence adjacent to and under the control of an effective eukaryotic promoter such as promoters used in combination with Chinese hamster ovary cells. To bring a coding sequence under control of a promoter, whether it is eukaryotic or prokaryotic, what is generally needed is to position the 5' end of the translation initiation side of the proper translational reading frame of the polypeptide between about 1 and about 50 nucleotides 3' of or downstream with respect to the promoter chosen. Furthermore, where eukaryotic expression is anticipated, one would typically desire to incorporate into the transcriptional unit which includes the peroxisome proliferator-activated receptor polypeptide, an appropriate polyadenylation site.

Incorporation of polynucleotides of the present invention into expression vectors can be achieved by a variety of techniques that will be recognized by those of ordinary skill in the art. For example, excised inserts of the hPPAR- $\gamma$  transcripts of the present invention can be blunt ended and ligated into blunt ended pcINEO (Introvigen) plasmids which contain the constitutively active CMV promoter, a multiple cloning site cassette flanked by T7 and SP6 promoters to ascertain directionality, as well as a NEO cassette to allow for neomycin selection. HeLa cell lines stably expressing the hPPAR- $\gamma$  polypeptides can be created by subcloning cDNA inserts into the pMV 7 and pMV 12 retroviral expression vectors. These vectors have been proven to be capable of generation of stable mammalian

cell lines in the following manner: pMV 7 and pMV 12 plasmids, each containing a hPPAR- $\gamma$  cDNA, are transfected by lipofection technique into  $\Psi$ 2 cells. Selection for cells expressing the virus-encoded gene is done after 48 hours with G418 (for pMV 7 transfections) or hygromycin B (for pMV 12 transfections). After one week of selection, surviving cells are pooled and used to produce ecotropic retrovirus, which is then used to infect the amphotropic producer cell line PA317, which will generate higher titer virus that can infect all mammalian cells. Selected PA317 cells can be used to produce virus, which in turn is used to infect Hela cells. After selection, resistant Hela cell clones are selected and expanded as clonal cell lines. Presence of hPPAR- $\gamma$  transcripts and polypeptides can be confirmed through western and northern blot analysis.

The pCMV plasmids are a series of mammalian expression vectors of particular utility in the present invention. The vectors are designed for use in essentially all cultured cells and work extremely well in SV40-transformed simian COS cell lines. The pCMV1, 2, 3, and 5 vectors differ from each other in certain unique restriction sites in the polylinker region of each plasmid. The pCMV4 vector differs from these 4 plasmids in containing a translation enhancer in the sequence prior to the polylinker. While they are not directly derived from the pCMV1-5 series of vectors, the functionally similar pCMV6b and c vectors are available from the Chiron Corp. of Emeryville, CA and are identical except for the orientation of the polylinker region which is reversed in one relative to the other.

The universal components of the pCMV plasmids are as follows. The vector backbone is pTZ18R (Pharmacia), and contains a bacteriophage  $\phi$ 1 origin of replication for production of single stranded DNA and an ampicillin-resistance gene. The CMV region consists of nucleotides -760 to +3 of the powerful promoter-regulatory region of the human cytomegalovirus (Towne strain) major immediate early gene (Thomsen *et al.*,



1984; Boshart *et al.*, 1985). The human growth hormone fragment (hGH) contains transcription termination and poly-adenylation signals representing sequences 1533 to 2157 of this gene (Seeburg, 1982). There is an *Alu* middle repetitive DNA sequence in this fragment. Finally, the SV40 origin of replication and early region promoter-enhancer derived from the pcD-X plasmid (*Hind*II to *Pst*I fragment) described in (Okayama *et al.*, 1983). The promoter in this fragment is oriented such that transcription proceeds away from the CMV/hGH expression cassette.

The pCMV plasmids are distinguishable from each other by differences in the polylinker region and by the presence or absence of the translation enhancer. The starting pCMV1 plasmid has been progressively modified to render an increasing number of unique restriction sites in the polylinker region. To create pCMV2, one of two *Eco*RI sites in pCMV1 were destroyed. To create pCMV3, pCMV1 was modified by deleting a short segment from the SV40 region (*Stu*I to *Eco*RI), and in so doing made unique the *Pst*I, *Sal*I, and *Bam*HI sites in the polylinker. To create pCMV4, a synthetic fragment of DNA corresponding to the 5'-untranslated region of a mRNA transcribed from the CMV promoter was added. The sequence acts as a translational enhancer by decreasing the requirements for initiation factors in polypeptide synthesis. To create pCMV5, a segment of DNA (*Hpa*I to *Eco*RI) was deleted from the SV40 origin region of pCMV1 to render unique all sites in the starting polylinker.

The pCMV vectors have been successfully expressed in simian COS cells, mouse L cells, CHO cells, and HeLa cells. In several side by side comparisons they have yielded 5- to 10-fold higher expression levels in COS cells than SV40-based vectors. The pCMV vectors have been used to express the LDL receptor, nuclear factor 1, G, alpha polypeptide, polypeptide phosphatase, synaptophysin, synapsin, insulin receptor, influenza hemagglutinin, androgen receptor, sterol 26-hydroxylase, steroid 17- and 21-

hydroxylase, cytochrome P-450 oxidoreductase, beta-adrenergic receptor, folate receptor, cholesterol side chain cleavage enzyme, and a host of other cDNAs. It should be noted that the SV40 promoter in these plasmids can be used to express other genes such as dominant selectable markers. Finally, there is an ATG sequence in the polylinker between the *Hind*III and *Pst*I sites in pCMU that can cause spurious translation initiation. This codon should be avoided if possible in expression plasmids. A paper describing the construction and use of the parenteral pCMV1 and pCMV4 vectors has been published.

#### IV. Transfected Cells.

In yet another embodiment, the present invention provides a recombinant host cell transfected with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide. Preferably, a recombinant host cell of the present invention is transfected with the polynucleotide of SEQ ID NO:1. Even more preferably, a host cell of the invention is a eukaryotic host cell. Still more preferably, a recombinant host cell of the present invention is a yeast cell. Alternatively, a recombinant host cell of the invention can be, by way of example and without limitation, a COS-1 cell, or a HeLa cell or a CV-1 cell. Means of transforming or transfecting cells with exogenous polynucleotide such as DNA molecules are well known in the art and include techniques such as calcium-phosphate- or DEAE-dextran-mediated transfection, protoplast fusion, electroporation, liposome mediated transfection, direct microinjection and adenovirus infection (Sambrook *et al.*, 1989).

*Saccharomyces cerevisiae* or common baker's yeast is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available. For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used (Stinchcomb *et al.*, 1979; Kingsman *et al.*, 1979; Tschemper *et al.*, 1980). This plasmid already

contains the *trp1* gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, 1977). The presence of the *trp1* lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoter sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, 1980) or other glycolytic enzymes (Hess *et al.*, 1968; Holland *et al.*, 1978) such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also introduced into the expression vector downstream from the sequences to be expressed to provide polyadenylation of the mRNA and termination. Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter region for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Any plasmid vector containing a yeast-compatible promoter, origin or replication and termination sequences is suitable.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* can be transformed

using pBR322, a plasmid derived from an *E. coli* species (Bolivar *et al.*, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR plasmid, or other microbial plasmid or phage must also contain, or be  
5 modified to contain, promoters which can be used by the microbial organism for expression of its own polypeptides.

Those promoters most commonly used in recombinant DNA construction include the  $\beta$ -lactamase (penicillinase) and lactose promoter systems (Chang *et al.*, 1978; Itakura *et al.*, 1977; Goeddel *et al.*, 1979; 10 Goeddel *et al.*, 1980) and a tryptophan (TRP) promoter system (EPO Appl. Publ. No. 0036776; Siebwenlist *et al.*, 1980). While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling a skilled worker to introduce functional promoters into  
15 plasmid vectors (Siebwenlist *et al.*, 1980).

In another aspect, a recombinant host cell of the present invention is a prokaryotic host cell. Preferably, a recombinant host cell of the invention is a bacterial cell of the DH5 $\alpha$  strain of *Escherichia coli*. More preferably, a recombinant host cell comprises a polynucleotide under the transcriptional  
20 control of regulatory signals functional in the recombinant host cell, wherein the regulatory signals appropriately control expression of a peroxisome proliferator-activated receptor polypeptide in a manner to enable all necessary transcriptional and post-transcriptional modification. The aforementioned strains, as well as *E. coli* W3110 (F<sup>-</sup>, lambda<sup>-</sup>, prototrophic, 25 ATCC No. 273325), bacilli such as *Bacillus subtilis*, or other enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcesans*, and various *Pseudomonas* species can be used.

The most widely used method is transfection mediated by either calcium phosphate or DEAE-dextran. Although the mechanism remains obscure, it is believed that the transfected DNA enters the cytoplasm of the cell by endocytosis and is transported to the nucleus. Depending on the cell type, up to 90% of a population of cultured cells can be transfected at any one time. Because of its high efficiency, transfection mediated by calcium phosphate or DEAE-dextran is the method of choice for experiments that require transient expression of the foreign DNA in large numbers of cells. Calcium phosphate-mediated transfection is also used to establish cell lines that integrate copies of the foreign DNA, which are usually arranged in head-to-tail tandem arrays into the host cell genome.

In the protoplast fusion method, protoplasts derived from bacteria carrying high numbers of copies of a plasmid of interest are mixed directly with cultured mammalian cells. After fusion of the cell membranes (usually with polyethylene glycol), the contents of the bacteria are delivered into the cytoplasm of the mammalian cells and the plasmid DNA is transported to the nucleus. Protoplast fusion is not as efficient as transfection for many of the cell lines that are commonly used for transient expression assays, but it is useful for cell lines in which endocytosis of DNA occurs inefficiently. Protoplast fusion frequently yields multiple copies of the plasmid DNA tandemly integrated into the host chromosome.

The application of brief, high-voltage electric pulses to a variety of mammalian and plant cells leads to the formation of nanometer-sized pores in the plasma membrane. DNA is taken directly into the cell cytoplasm either through these pores or as a consequence of the redistribution of membrane components that accompanies closure of the pores. Electroporation can be extremely efficient and can be used both for transient expression of cloned genes and for establishment of cell lines that carry integrated copies of the gene of interest. Electroporation, in contrast to

calcium phosphate-mediated transfection and protoplast fusion, frequently gives rise to cell lines that carry one, or at most a few, integrated copies of the foreign DNA.

Liposome transfection involves encapsulation of DNA and RNA within liposomes, followed by fusion of the liposomes with the cell membrane. The mechanism of how DNA is delivered into the cell is unclear but transfection efficiencies can be as high as 90%.

Direct microinjection of a DNA molecule into nuclei has the advantage of not exposing DNA to cellular components such as low-pH endosomes. Microinjection is therefore used primarily as a method to establish lines of cells that carry integrated copies of the DNA of interest.

The use of adenovirus as a vector for cell transfection is well known in the art. Adenovirus vector-mediated cell transfection has been reported for various cells (Stratford-Perricaudet *et al.*, 1992).

In addition to microorganisms, cultures of cells derived from multicellular organisms can also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years (Kruse and Peterson, 1973). Examples of such useful host cell lines are AtT-20, VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COSM6, COS-7, 293 and MDCK cell lines. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located upstream of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences.

For use in mammalian cells, the control functions on the expression vectors are often derived from viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, Cytomegalovirus and most frequently Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers *et al.*, 1978). Smaller or larger SV40 fragments can also be used, provided there is included the approximately 250 bp sequence extending from the *Hind*III site toward the *Bgl*I site located in the viral origin of replication. Further, it is also possible, and often desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

An origin of replication can be provided with by construction of the vector to include an exogenous origin, such as can be derived from SV40 or other viral (e.g., Polyoma, Adeno, VSV, BPV, CMV) source, or can be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

V. Preparing Recombinant Peroxisome  
Proliferator-Activated Receptor Polypeptides.

In yet another embodiment, the present invention contemplates a process of preparing a peroxisome proliferator-activated receptor polypeptide comprising transfecting a cell with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide to produce a transformed host cell; and maintaining the transformed host cell under biological conditions sufficient for expression of the polypeptide. Preferably, the transformed host cell is a eukaryotic cell. More preferably still, the eukaryotic cell is a COS-1 cell. Alternatively, the host cell is a prokaryotic cell. More preferably, the prokaryotic cell is a bacterial cell of the DH5 $\alpha$  strain of *Escherichia coli*. Even more preferably, a polynucleotide transfected into the transformed cell comprises the nucleotide base sequence of SEQ ID NO:1.

A host cell used in the process is capable of expressing a functional, recombinant peroxisome proliferator-activated receptor polypeptide. A preferred host cell is a Chinese hamster ovary cell. However, a variety of cells are amenable to a process of the invention, for instance, yeasts cells, human cell lines, and other eukaryotic cell lines known well to those of skill in the art.

Following transfection, the cell is maintained under culture conditions for a period of time sufficient for expression of a peroxisome proliferator-activated receptor polypeptide. Culture conditions are well known in the art and include ionic composition and concentration, temperature, pH and the like. Typically, transfected cells are maintained under culture conditions in a culture medium. Suitable medium for various cell types are well known in the art. In a preferred embodiment, temperature is from about 20°C to about 50°C, more preferably from about 30°C to about 40°C and, even more preferably about 37°C.



pH is preferably from about a value of 6.0 to a value of about 8.0, more preferably from about a value of about 6.8 to a value of about 7.8 and, most preferably about 7.4. Osmolality is preferably from about 200 milliosmols per liter (mosm/L) to about 400 mosm/l and, more preferably  
5 from about 290 mosm/L to about 310 mosm/L. Other biological conditions needed for transfection and expression of an encoded protein are well known in the art.

Transfected cells are maintained for a period of time sufficient for expression of a peroxisome proliferator-activated receptor polypeptide. A  
10 suitable time depends *inter alia* upon the cell type used and is readily determinable by a skilled artisan. Typically, maintenance time is from about 2 to about 14 days.

Recombinant peroxisome proliferator-activated receptor polypeptide is recovered or collected either from the transfected cells or the medium in  
15 which those cells are cultured. Recovery comprises isolating and purifying the peroxisome proliferator-activated receptor polypeptide. Isolation and purification techniques for polypeptides are well known in the art and include such procedures as precipitation, filtration, chromatography, electrophoresis and the like.

## 20 VI. Antibodies and Methods.

In still another embodiment, the present invention provides an antibody immunoreactive with a peroxisome proliferator-activated receptor polypeptide. Preferably, an antibody of the invention is a monoclonal antibody. More preferably, the antibody is immunoreactive with a  
25 peroxisome proliferator-activated receptor polypeptide that comprises the amino acid residue sequence of SEQ ID NO:2. Means for preparing and characterizing antibodies are well known in the art (*See, e.g., Antibodies "A*

*Laboratory Manual*, " E. Howell and D. Lane, Cold Spring Harbor Laboratory, 1988).

Briefly, a polyclonal antibody is prepared by immunizing an animal with an immunogen comprising a polypeptide or polynucleotide of the present invention, and collecting antisera from that immunized animal. A wide range of animal species can be used for the production of antisera. Typically an animal used for production of anti-antisera is a rabbit, a mouse, a rat, a hamster or a guinea pig. Because of the relatively large blood volume of rabbits, a rabbit is a preferred choice for production of polyclonal antibodies.

As is well known in the art, a given polypeptide or polynucleotide may vary in its immunogenicity. It is often necessary therefore to couple the immunogen (e.g., a polypeptide or polynucleotide) of the present invention with a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin can also be used as carriers.

Means for conjugating a polypeptide or a polynucleotide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine.

As is also well known in the art, immunogenicity to a particular immunogen can be enhanced by the use of non-specific stimulators of the immune response known as adjuvants. Exemplary and preferred adjuvants include complete Freund's adjuvant, incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

The amount of immunogen used of the production of polyclonal antibodies varies *inter alia*, upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal. The production of polyclonal antibodies is monitored by sampling blood of the immunized animal at various points following immunization. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored.

In another aspect, the present invention contemplates a process of producing an antibody immunoreactive with a peroxisome proliferator-activated receptor polypeptide comprising the steps of (a) transfecting a recombinant host cell with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide; (b) culturing the host cell under conditions sufficient for expression of the polypeptide; (c) recovering the polypeptide; and (d) preparing the antibody to the polypeptide. Preferably, the host cell is transfected with the polynucleotide of SEQ ID NO:1. Even more preferably, the present invention provides an antibody prepared according to the process described above.

A monoclonal antibody of the present invention can be readily prepared through use of well-known techniques such as those exemplified in U.S. Pat. No 4,196,265, herein incorporated by reference. Typically, a technique involves first immunizing a suitable animal with a selected antigen (e.g., a polypeptide or polynucleotide of the present invention) in a manner sufficient to provide an immune response. Rodents such as mice and rats are preferred animals. Spleen cells from the immunized animal are then fused with cells of an immortal myeloma cell. Where the immunized animal is a mouse, a preferred myeloma cell is a murine NS-1 myeloma cell.

The fused spleen/myeloma cells are cultured in a selective medium to select fused spleen/myeloma cells from the parental cells. Fused cells are separated from the mixture of non-fused parental cells, for example, by the addition of agents that block the *de novo* synthesis of nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. Aminopterin and methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides. Where azaserine is used, the media is supplemented with hypoxanthine.

This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants for reactivity with an antigen-polypeptide. The selected clones can then be propagated indefinitely to provide the monoclonal antibody.

By way of specific example, to produce an antibody of the present invention, mice are injected intraperitoneally with between about 1-200  $\mu\text{g}$  of an antigen comprising a polypeptide of the present invention. B lymphocyte cells are stimulated to grow by injecting the antigen in association with an adjuvant such as complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*). At some time (e.g., at least two weeks) after the first injection, mice are boosted by injection with a second dose of the antigen mixed with incomplete Freund's adjuvant.

A few weeks after the second injection, mice are tail bled and the sera titered by immunoprecipitation against radiolabeled antigen. Preferably, the process of boosting and titering is repeated until a suitable titer is

achieved. The spleen of the mouse with the highest titer is removed and the spleen lymphocytes are obtained by homogenizing the spleen with a syringe. Typically, a spleen from an immunized mouse contains approximately  $5 \times 10^7$  to  $2 \times 10^8$  lymphocytes.

5                    Mutant lymphocyte cells known as myeloma cells are obtained from laboratory animals in which such cells have been induced to grow by a variety of well-known methods. Myeloma cells lack the salvage pathway of nucleotide biosynthesis. Because myeloma cells are tumor cells, they can be propagated indefinitely in tissue culture, and are thus denominated immortal.

10                  Numerous cultured cell lines of myeloma cells from mice and rats, such as murine NS-1 myeloma cells, have been established.

                    Myeloma cells are combined under conditions appropriate to foster fusion with the normal antibody-producing cells from the spleen of the mouse or rat injected with the antigen/polypeptide of the present invention.

15                  Fusion conditions include, for example, the presence of polyethylene glycol. The resulting fused cells are *hybridoma* cells. Like myeloma cells, hybridoma cells grow indefinitely in culture.

                    Hybridoma cells are separated from unfused myeloma cells by culturing in a selection medium such as HAT media (hypoxanthine, aminopterin, thymidine). Unfused myeloma cells lack the enzymes necessary to synthesize nucleotides from the salvage pathway because they are killed in the presence of aminopterin, methotrexate, or azaserine.

20                  Unfused lymphocytes also do not continue to grow in tissue culture. Thus, only cells that have successfully fused (hybridoma cells) can grow in the selection media.

25

                    Each of the surviving hybridoma cells produces a single type of antibody. These cells are then screened for the production of the specific

antibody immunoreactive with an antigen/polypeptide of the present invention. Single cell hybridomas are isolated by limiting dilutions of the hybridomas. The hybridomas are serially diluted many times and, after the dilutions are allowed to grow, the supernatant is tested for the presence of the monoclonal antibody. The clones producing that antibody are then cultured in large amounts to produce an antibody of the present invention in convenient quantity.

#### VII. Detecting Polynucleotides and the Polypeptides Encoded.

By use of a monoclonal antibody of the present invention, specific polypeptides and polynucleotide of the invention can be recognized as antigens, and thus identified. Once identified, those polypeptides and polynucleotide can be isolated and purified by techniques such as *antibody-affinity chromatography*. In antibody-affinity chromatography, a monoclonal antibody is bound to a solid substrate and exposed to a solution containing the desired antigen. The antigen is removed from the solution through an immunospecific reaction with the bound antibody. The polypeptide or polynucleotide is then easily removed from the substrate and purified.

The present invention thus provides a process of detecting a peroxisome proliferator-activated receptor polypeptide, wherein the process comprises immunoreacting the polypeptide with an antibody prepared according to the process described above to form an antibody-polypeptide conjugate, and detecting the conjugate.

In yet another embodiment, the present invention contemplates a process of detecting a messenger RNA transcript that encodes a peroxisome proliferator-activated receptor polypeptide, wherein the process comprises (a) hybridizing the messenger RNA transcript with a polynucleotide sequence that encodes the peroxisome proliferator-activated receptor polypeptide to form a duplex; and (b) detecting the duplex. Alternatively, the present

invention provides a process of detecting a DNA molecule that encodes a peroxisome proliferator-activated receptor polypeptide, wherein the process comprises (a) hybridizing DNA molecules with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide to form a duplex; and (b) detecting the duplex.

#### VIII. Pharmaceutical Compositions.

Alternatively, the present invention contemplates a pharmaceutical composition comprising a peroxisome proliferator-activated receptor polypeptide and a physiologically acceptable carrier. Preferably, the present invention provides a pharmaceutical composition comprising a receptor polypeptide that comprises the amino acid residue sequence of SEQ ID NO:2. In another embodiment, the present invention provides a pharmaceutical composition comprising a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide, and a physiologically acceptable carrier. Preferably, that pharmaceutical composition comprises a polynucleotide encoding a receptor polypeptide comprising the amino acid residue sequence of SEQ ID NO:2. Even more preferably, the pharmaceutical composition comprises a polynucleotide that comprises the nucleotide sequence of SEQ ID NO:1.

A composition of the present invention is typically administered parenterally in dosage unit formulations containing standard, well-known nontoxic physiologically acceptable carriers, adjuvants, and vehicles as desired. The term parenteral as used herein includes intravenous, intramuscular, intraarterial injection, or infusion techniques.

Injectable preparations, for example sterile injectable aqueous or oleaginous suspensions, are formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension

in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol.

Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Preferred carriers include neutral saline solutions buffered with phosphate, lactate, Tris, and the like. Of course, one purifies the vector sufficiently to render it essentially free of undesirable contaminants, such as defective interfering adenovirus particles or endotoxins and other pyrogens such that it does not cause any untoward reactions in the individual receiving the vector construct. A preferred means of purifying the vector involves the use of buoyant density gradients, such as cesium chloride gradient centrifugation.

A carrier can also be a liposome. Means for using liposomes as delivery vehicles are well known in the art (See, e.g. Gabizon *et al.*, 1990; Ferruti *et al.*, 1986; and Ranade, 1989).

A transfected cell can also serve as a carrier. By way of example, a liver cell can be removed from an organism, transfected with a polynucleotide of the present invention using methods set forth above and then the transfected cell returned to the organism (e.g. injected intravascularly).

#### IX. Screening Assays.

In yet another aspect, the present invention contemplates a process of screening substances for their ability to interact with a peroxisome



proliferator-activated receptor polypeptide comprising the steps of providing a peroxisome proliferator-activated receptor polypeptide, and testing the ability of selected substances to interact with the peroxisome proliferator-activated receptor polypeptide. In a preferred embodiment, providing a  
5 peroxisome proliferator-activated receptor polypeptide is transfecting a host cell with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide to form a transformed cell and maintaining the transformed cell under biological conditions sufficient for expression of the peroxisome proliferator-activated receptor polypeptide.

10 Utilizing the methods and compositions of the present invention, screening assays for the testing of candidate substances such as agonists and antagonists of peroxisome proliferator-activated receptors can be derived. A candidate substance is a substance which potentially can interact with or modulate, by binding or other intramolecular interaction, a peroxisome  
15 proliferator-activated receptor polypeptide. In some instances, such a candidate substance will be an agonist of the receptor and in other instances can exhibit antagonistic attributes when interacting with the receptor polypeptide. In other instances, such substances can have mixed agonistic and antagonistic properties or can modulate the peroxisome proliferator-  
20 activated receptor in other ways.

Cloned expression systems such as those of the present invention are useful where there is difficulty in obtaining tissue that satisfactorily expresses a particular receptor. Cost is another very real advantage, at least with regard to the microbial expression systems of the present invention. For  
25 antagonists in a primary screen, microorganism expression systems of the present invention are inexpensive in comparison to prior art tissue-screening methods.

With the availability of cloned receptors, recombinant receptor screening systems have several advantages over tissue based systems. A major advantage is that the investigator can now control the type of receptor that is utilized in a screening assay. Specific receptor sub-types and sub-sub-types can be preferentially expressed and its interaction with a ligand can be identified. Other advantages include the availability of large amounts of receptor, the availability of rare receptors previously unavailable in tissue samples, and the lack of expenses associated with the maintenance of live animals.

Screening assays of the present invention generally involve determining the ability of a candidate substance to bind to the receptor and to affect the activity of the receptor, such as the screening of candidate substances to identify those that inhibit or otherwise modify the receptor's function. Typically, this method includes preparing recombinant receptor polypeptide, followed by testing the recombinant polypeptide or cells expressing the polypeptide with a candidate substance to determine the ability of the substance to affect its physiological function. In preferred embodiments, the invention relates to the screening of candidate substances to identify those that affect the transcription regulation activity of the human receptor, and thus can be suitable for use in humans.

As is well known in the art, a screening assay provides a receptor under conditions suitable for the binding of an agent to the receptor. These conditions include but are not limited to pH, temperature, tonicity, the presence of relevant co-factors, and relevant modifications to the polypeptide such as glycosylation or prenylation. It is contemplated that the receptor can be expressed and utilized in a prokaryotic or eukaryotic cell. The host cell expressing the receptor can be used whole or the receptor can be isolated from the host cell. The host cell can also be fractionated into sub-cellular fractions where the receptor can be found. For example, cells expressing the

receptor can be fractionated into the nuclei, the endoplasmic reticulum, vesicles, or the membrane surfaces of the cell.

pH is preferably from about a value of 6.0 to a value of about 8.0, more preferably from about a value of about 6.8 to a value of about 7.8 and, most preferably about 7.4. In a preferred embodiment, temperature is from about 20°C to about 50°C, more preferably from about 30°C to about 40°C and, even more preferably about 37°C. Osmolality is preferably from about 5 milliosmols per liter (mosm/L) to about 400 mosm/l and, more preferably from about 200 milliosmols per liter to about 400 mosm/l and, even more preferably from about 290 mosm/L to about 310 mosm/L. The presence of co-factors can be required for the proper functioning of the receptor. Typical co-factors include sodium, potassium, calcium, magnesium, and chloride. In addition, small, non-peptide molecules, known as prosthetic groups can be required. Other biological conditions needed for receptor function are well known in the art.

It is well known in the art that proteins can be reconstituted in artificial membranes, vesicles or liposomes (Danboldt *et al.*, 1990). The present invention contemplates that the receptor can be incorporated into artificial membranes, vesicles or liposomes. The reconstituted receptor can be utilized in screening assays.

It is further contemplated that the receptor of the present invention can be coupled to a solid support. The solid support can be agarose beads, polyacrylamide beads, polyacrylic beads or other solid matrices capable of being coupled to proteins. Well known coupling agents include cyanogen bromide, carbonyldiimidazole, tosyl chloride, and gluteraldehyde.

It is further contemplated that secondary polypeptides which can function in conjunction with the receptor of the present invention can be

provided. For example, the receptor of the present invention exerts its physiological effects in conjunction with a G-protein and an effector polypeptide.

In a typical screening assay for identifying candidate substances, one  
5 employs the same recombinant expression host as the starting source for obtaining the receptor polypeptide, generally prepared in the form of a crude homogenate. Recombinant cells expressing the receptor are washed and homogenized to prepare a crude polypeptide homogenate in a desirable buffer such as disclosed herein. In a typical assay, an amount of polypeptide  
10 from the cell homogenate, is placed into a small volume of an appropriate assay buffer at an appropriate pH. Candidate substances are added to the admixture in convenient concentrations and the interaction between the candidate substance and the receptor polypeptide is monitored.

Where one uses an appropriate known substrate for the receptor, one  
15 can, in the foregoing manner, obtain a baseline activity for the recombinantly produced receptor. Then, to test for inhibitors or modifiers of the receptor function, one can incorporate into the admixture a candidate substance whose effect on the receptor is unknown. By comparing reactions which are carried out in the presence or absence of the candidate substance,  
20 one can then obtain information regarding the effect of candidate/receptor interaction on the receptor's normal function of regulation of a reporter construct.

Accordingly, it is proposed that this aspect of the present invention provides those of skill in the art with methodology that allows for the  
25 identification of candidate substances having the ability to modify the action of peroxisome proliferator-activated receptor polypeptides in one or more manners.

Additionally, screening assays for the testing of candidate substances are designed to allow the investigation of structure/activity relationships of ligands with the receptors, e.g., study of binding of naturally occurring hormones or other substances capable of interacting or otherwise modulating with the receptor versus studies of the activity caused by the binding of such molecules to the receptor. In certain embodiments, the polypeptides of the invention are crystallized in order to carry out x-ray crystallographic studies as a means of evaluating interactions with candidate substances or other molecules with the peroxisome proliferator-activated receptor polypeptide. For instance, the purified recombinant polypeptides of the invention, when crystallized in a suitable form, are amenable to detection of intra-molecular interactions by x-ray crystallography.

An important aspect of the invention is the use of recombinantly produced peroxisome proliferator-activated receptor polypeptide in screening assays for the identification of substances which can inhibit or otherwise modify or alter the function of the receptor. The use of recombinantly produced receptor is of particular benefit because the naturally occurring receptor is present in only small quantities and has proven difficult to purify. Moreover, this provides a ready source of receptor, which has heretofore been unavailable.

As described above, receptors in the presence of agonists exert their physiological effects through a secondary molecule. A screening assay of the invention, in preferred embodiments, conveniently employs a peroxisome proliferator-activated receptor polypeptide directly from the recombinant host in which it is produced. This is achieved most preferably by simply expressing the selected polypeptide within the recombinant host, typically a eukaryotic host, followed by preparing a crude homogenate which includes the enzyme. A portion of the crude homogenate is then admixed with an appropriate effector of the receptor along with the candidate substance to be

tested. By comparing the binding of the selected effector to the receptor in the presence or absence of the candidate substance, one can obtain information regarding the physiological properties of the candidate substance.

The receptor can be expressed in a prokaryotic or a eukaryotic cell.  
5 Receptors have been expressed in *E. coli* (Bertin *et al.*, 1992), in yeast (King *et al.*, 1990) and in mammalian cells (Bouvier *et al.*, 1988).

A cell expressing a receptor can be used whole to screen agents. For example, cells expressing the receptor of the present invention can be exposed to radiolabelled agent and the amount of binding of the radiolabelled agent to the cell can be determined.  
10

The cell expressing the receptor can be fractionated into sub-cellular components which contain the receptor of the present invention. Methods for purifying sub-cellular fractions are well known in the art. Sub-cellular fractions include but are not limited to the cytoplasm, cellular membrane, other membranous fractions such as the endoplasmic reticulum, golgi bodies, vesicles and the nucleus. Receptors isolated as sub-cellular fractions can be associated with cellular membranes. For example, if cellular membrane vesicles are isolated from the cell expressing the receptor, the receptor molecule can be membrane bound. It is further contemplated that the  
15  
20 receptor of the present invention can be purified from a cell that expresses the receptor. Methods of purification are well known in the art. The purified receptor can be used in screening assays.

There are believed to be a wide variety of embodiments which can be employed to determine the effect of the candidate substance on the receptor polypeptides of the invention, and the invention is not intended to be limited to any one such method. However, it is generally desirable to employ a system wherein one can measure the ability of the receptor polypeptide to  
25

bind to and or be modified by the effector employed in the presence of a particular substance.

The detection of an interaction between an agent and a receptor can be accomplished through techniques well known in the art. These techniques include but are not limited to centrifugation, chromatography, electrophoresis and spectroscopy. The use of isotopically labelled reagents in conjunction with these techniques or alone is also contemplated. Commonly used radioactive isotopes include  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{22}\text{Na}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{45}\text{Ca}$ ,  $^{60}\text{Co}$ ,  $^{125}\text{I}$ , and  $^{131}\text{I}$ . Commonly used stable isotopes include  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{18}\text{O}$ .

For example, if an agent can bind to the receptor of the present invention, the binding can be detected by using radiolabelled agent or radiolabelled receptor. Briefly, if radiolabelled agent or radiolabelled receptor is utilized, the agent-receptor complex can be detected by liquid scintillation or by exposure to X-Ray film.

When an agent modifies the receptor, the modified receptor can be detected by differences in mobility between the modified receptor and the unmodified receptor through the use of chromatography, electrophoresis or centrifugation. When the technique utilized is centrifugation, the differences in mobility is known as the sedimentation coefficient. The modification can also be detected by differences between the spectroscopic properties of the modified and unmodified receptor. As a specific example, if an agent covalently modifies a receptor, the difference in retention times between modified and unmodified receptor on a high pressure liquid chromatography (HPLC) column can easily be detected.

As a specific example, if an agent covalently modifies a receptor, the spectroscopic differences between modified and unmodified receptor in the nuclear magnetic resonance (NMR) spectra can be detected. Alternatively,

one can focus on the agent and detect the differences in the spectroscopic properties or the difference in mobility between the free agent and the agent after modification of the receptor.

5                   When a secondary polypeptide is provided, the agent-receptor-secondary polypeptide complex or the receptor-secondary polypeptide complex can be detected. Differences in mobility or differences in spectroscopic properties as described above can be detected.

10                   It is further contemplated that when a secondary polypeptide is provided the enzymatic activity of the effector polypeptide can be detected. For example, many receptors exert physiological effects through the stimulation or inhibition of adenylyl cyclase. The enzymatic activity of adenylyl cyclase in the presence of an agent can be detected.

15                   The interaction of an agent and a receptor can be detected by providing a reporter gene. Well known reporter genes include  $\beta$ -galactosidase ( $\beta$ -Gal), chloramphenicol transferase (CAT) and luciferase. The reporter gene is expressed by the host and the enzymatic reaction of the reporter gene product can be detected.

20                   In preferred assays, an admixture containing the polypeptide, effector and candidate substance is allowed to incubate for a selected amount of time, and the resultant incubated mixture subjected to a separation means to separate the unbound effector remaining in the admixture from any effector/receptor complex so produced. Then, one simply measures the amount of each (e.g., versus a control to which no candidate substance has been added). This measurement can be made at various time points where  
25                   velocity data is desired. From this, one can determine the ability of the candidate substance to alter or modify the function of the receptor.



Numerous techniques are known for separating the effector from effector/receptor complex, and all such methods are intended to fall within the scope of the invention. Use of thin layer chromatographic methods (TLC), HPLC, spectrophotometric, gas chromatographic/mass spectrophotometric or NMR analyses. It is contemplated that any such technique can be employed so long as it is capable of differentiating between the effector and complex, and can be used to determine enzymatic function such as by identifying or quantifying the substrate and product.

A. Screening assays for peroxisome  
proliferator-activated receptor polypeptides.

The present invention provides a process of screening a biological sample for the presence of a peroxisome proliferator-activated receptor polypeptide. A biological sample to be screened can be a biological fluid such as extracellular or intracellular fluid or a cell or tissue extract or homogenate. A biological sample can also be an isolated cell (e.g., in culture) or a collection of cells such as in a tissue sample or histology sample. A tissue sample can be suspended in a liquid medium or fixed onto a solid support such as a microscope slide.

In accordance with a screening assay process, a biological sample is exposed to an antibody immunoreactive with the peroxisome proliferator-activated receptor polypeptide whose presence is being assayed. Typically, exposure is accomplished by forming an admixture in a liquid medium that contains both the antibody and the candidate peroxisome proliferator-activated receptor polypeptide. Either the antibody or the sample with the peroxisome proliferator-activated receptor polypeptide can be affixed to a solid support (e.g., a column or a microtiter plate).

The biological sample is exposed to the antibody under biological reaction conditions and for a period of time sufficient for antibody-

polypeptide conjugate formation. Biological reaction conditions include ionic composition and concentration, temperature, pH and the like.

5 Ionic composition and concentration can range from that of distilled water to a 2 molal solution of NaCl. Preferably, osmolality is from about 100 mosmols/l to about 400 mosmols/l and, more preferably from about 200 mosmols/l to about 300 mosmols/l. Temperature preferably is from about 4°C to about 100°C, more preferably from about 15°C to about 50°C and, even more preferably from about 25°C to about 40°C. pH is preferably from about a value of 4.0 to a value of about 9.0, more preferably from about a value of 6.5 to a value of about 8.5 and, even more preferably from about a value of 7.0 to a value of about 7.5. The only limit on biological reaction conditions is that the conditions selected allow for antibody-polypeptide conjugate formation and that the conditions do not adversely affect either the antibody or the peroxisome proliferator-activated receptor polypeptide.

10 Exposure time will vary *inter alia* with the biological conditions used, the concentration of antibody and polypeptide and the nature of the sample (e.g., fluid or tissue sample). Means for determining exposure time are well known to one of ordinary skill in the art. Typically, where the sample is fluid and the concentration of polypeptide in that sample is about  $10^{-10}$  M, exposure time is from about 10 minutes to about 200 minutes.

25 The presence of peroxisome proliferator-activated receptor polypeptide in the sample is detected by detecting the formation and presence of antibody-peroxisome proliferator-activated receptor polypeptide conjugates. Means for detecting such antibody-antigen (e.g., receptor polypeptide) conjugates or complexes are well known in the art and include such procedures as centrifugation, affinity chromatography and the like, binding of a secondary antibody to the antibody-candidate receptor complex.

In one embodiment, detection is accomplished by detecting an indicator affixed to the antibody. Exemplary and well known such indicators include radioactive labels (e.g.,  $^{32}\text{P}$ ,  $^{125}\text{I}$ ,  $^{14}\text{C}$ ), a second antibody or an enzyme such as horse radish peroxidase. Means for affixing indicators to antibodies are well known in the art. Commercial kits are available.

B. Screening assay for anti-peroxisome  
proliferator-activated receptor antibody.

In another aspect, the present invention provides a process of screening a biological sample for the presence of antibodies immunoreactive with a peroxisome proliferator-activated receptor polypeptide (i.e., an anti-peroxisome proliferator-activated receptor antibody). In accordance with such a process, a biological sample is exposed to a peroxisome proliferator-activated receptor polypeptide under biological conditions and for a period of time sufficient for antibody-polypeptide conjugate formation and the formed conjugates are detected.

C. Screening assay for polynucleotide that encodes a  
peroxisome proliferator-activated receptor polypeptide.

A DNA molecule and, particularly a probe molecule, can be used for hybridizing as oligonucleotide probes to a DNA source suspected of possessing a peroxisome proliferator-activated receptor polypeptide encoding polynucleotide or gene. The probing is usually accomplished by hybridizing the oligonucleotide to a DNA source suspected of possessing such a receptor gene. In some cases, the probes constitute only a single probe, and in others, the probes constitute a collection of probes based on a certain amino acid sequence or sequences of the peroxisome proliferator-activated receptor polypeptide and account in their diversity for the redundancy inherent in the genetic code.

A suitable source of DNA for probing in this manner is capable of expressing peroxisome proliferator-activated receptor polypeptides and can

be a genomic library of a cell line of interest. Alternatively, a source of DNA can include total DNA from the cell line of interest. Once the hybridization process of the invention has identified a candidate DNA segment, one confirms that a positive clone has been obtained by further hybridization, restriction enzyme mapping, sequencing and/or expression and testing.

Alternatively, such DNA molecules can be used in a number of techniques including their use as: (1) diagnostic tools to detect normal and abnormal DNA sequences in DNA derived from patient's cells; (2) means for detecting and isolating other members of the peroxisome proliferator-activated receptor family and related polypeptides from a DNA library potentially containing such sequences; (3) primers for hybridizing to related sequences for the purpose of amplifying those sequences; (4) primers for altering the native peroxisome proliferator-activated receptor DNA sequences; as well as other techniques which rely on the similarity of the DNA sequences to those of the peroxisome proliferator-activated receptor DNA segments herein disclosed.

As set forth above, in certain aspects, DNA sequence information provided by the invention allows for the preparation of relatively short DNA (or RNA) sequences (e.g., probes) that specifically hybridize to encoding sequences of the selected peroxisome proliferator-activated receptor gene. In these aspects, nucleic acid probes of an appropriate length are prepared based on a consideration of the selected peroxisome proliferator-activated receptor sequence (e.g., a sequence such as that shown in SEQ ID NO: 1). The ability of such nucleic acid probes to specifically hybridize to peroxisome proliferator-activated receptor encoding sequences lend them particular utility in a variety of embodiments. Most importantly, the probes can be used in a variety of assays for detecting the presence of complementary sequences in a given sample. However, uses are envisioned,

including the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

In certain embodiments, it is advantageous to employ a nucleic acid sequence of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In preferred embodiments, one likely employs an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the case of enzyme tags, calorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples.

#### X. Screening Assay Kits.

In another aspect, the present invention contemplates a diagnostic assay kit for detecting the presence of a peroxisome proliferator-activated receptor polypeptide in a biological sample, where the kit comprises a first container containing a first antibody capable of immunoreacting with a peroxisome proliferator-activated receptor polypeptide, with the first antibody present in an amount sufficient to perform at least one assay. Preferably, an assay kit of the invention further comprises a second container containing a second antibody that immunoreacts with the first antibody. More preferably, the antibodies used in an assay kit of the present invention are monoclonal antibodies. Even more preferably, the first antibody is affixed to a solid support. More preferably still, the first and second antibodies comprise an indicator, and, preferably, the indicator is a radioactive label or an enzyme.

In an alternative aspect, the present invention provides a diagnostic assay kit for detecting the presence, in biological samples, of a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide, the kits comprising a first container that contains a second  
5 polynucleotide identical or complementary to a segment of at least 10 contiguous nucleotide bases of SEQ ID NO:1.

In another embodiment, the present invention contemplates a diagnostic assay kit for detecting the presence, in a biological sample, of an antibody immunoreactive with a peroxisome proliferator-activated receptor  
10 polypeptide, the kit comprising a first container containing a peroxisome proliferator-activated receptor polypeptide that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay.

### EXAMPLES

15 Examples have been included to illustrate preferred modes of the invention. Certain aspects of the following examples are described in terms of techniques and procedures found or contemplated by the present inventors to work well in the practice of the invention. These examples are exemplified through the use of standard laboratory practices of the inventor.  
20 In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following examples are intended to be exemplary only and that numerous changes, modifications and alterations can be employed without departing from the spirit and scope of the invention.

**Example 1:**            Cloning and sequence of human PPAR- $\gamma$ .

Generation and screening of human bone marrow cDNA library.

Oligo(dT)-primed first strand cDNA was prepared from 2.6  $\mu$ g poly  
A selected normal human bone marrow aspirate RNA (see below). The  
5        oligo (dT) primer  
(5'ACTAGTGC GGCCGCTAGGCCTCGAGT TTT TTT TTT TTT TTT 3')  
(SEQ ID NO:3) (Blumberg *et al.*, 1992) was used to create an oriented  
library, by a modified method of Blumberg (Blumberg *et al.*, 1992).  
5-methyl dCTP was incorporated during first strand synthesis to protect  
10       internal Xho 1 sites by hemi-methylation interference. Residual 5 methyl  
dCTPs were removed and second strand synthesis was performed,  
incorporating the 3' Xho 1 site. Internal EcoRI sites were then protected by  
EcoRI methylase treatment, the cDNA ends were made blunt-ended, and  
EcoRI linkers were added. After combined EcoRI and Xho 1 digestion, the  
15       cDNA was purified by Sepharose CL-4B chromatography to remove linkers  
and size fractionate the cDNA. Double stranded cDNAs, approximately 500  
base pairs or greater, were ligated to  $\lambda$  ZAP II arms (Stratagene) and  
packaged *in vitro* with Gigapack II Gold (Stratagene). This library contained  
7.4 x 10<sup>7</sup> independent clones and was screened unamplified.

20            The library was screened in duplicate with an HPLC-purified <sup>32</sup>P-  
labeled synthetic 512 fold-degenerate oligonucleotide  
(TGYGARGGNTGYAARGGNTTYTT) (SEQ ID NO:4) (Blumberg *et al.*,  
1992) under low stringency conditions (1M NaCl, 0.1M Tris-HCl pH 8.0, 6  
mM EDTA, 125 units of heparin per ml, 0.05% sodium dodecyl sulfate, at  
25       46C) and washed at high stringency (two 15 minute room temperature  
washes, one in 6XSSC/0.05% sodium pyrophosphate, and one in 3 M  
tetramethylammonium chloride/0.05 M Tris-HCl pH 8.0/0.2 mM EDTA  
(3M TMAC), two 20 minute washes at 58 C in 3 M TMAC, then one 15  
minute wash at room temperature in 6XSSC/0.05% sodium pyrophosphate).

This oligonucleotide (Blumberg *et al.*, 1992) is a mixture of all possible DNA sequences encoding the highly conserved amino acid sequence CEGCKGFF (SEQ ID NO:5), found in the first cysteine finger of the members of the thyroid-retinoid branch of the nuclear receptor superfamily (Wahli and Martinez, 1991; Evans, 1988). Positive plaques from the initial high density screen of  $1 \times 10^6$  clones were purified on secondary and tertiary screens, then converted to plasmids by the automatic excision process (Stratagene Unizap) (Short *et al.*, 1988). Purified plasmids were sequenced using a modified dideoxy chain termination method (Tabor and Richardson, 1989), and the screening oligonucleotide as the initial primer. Clones identified as either similar to or members of the thyroid retinoid branch of the nuclear receptor superfamily were further characterized.

DNA sequences were analyzed using programs of Staden (Staden, 1986), University of Wisconsin Genetics Computer Group (Devereaux, 1984), Feng and Doolittle (Feng and Doolittle, 1987), DNASIS (Hitachi), GeneWorks release 2.2 (IntelliGenetics) and DNA Strider release 1.0 (Commissariat a l'Energie Atomique, France).

Northern hybridizations: Prehybridizations and hybridizations were performed in 7% SDS, 0.5 M  $\text{Na}_2\text{PO}_4$  pH 7.2 with 100  $\mu\text{g}/\text{ml}$  salmon sperm DNA at high stringency (65 C) [15]. Double stranded, denatured cDNA fragments obtained by band preparation from agarose gels, ranging in size from 400-1600 bp were used as probes. These probes were random prime labeled with  $\text{P}^{32}$   $\alpha$  dCTP, with specific activities from  $0.4\text{-}1.0 \times 10^9$  cpm/ $\mu\text{g}$  (Pharmacia Oligolabelling kit), and used at concentrations of  $3$  to  $5 \times 10^6$  cpm per ml of hybridization solution. Exposures ranged from overnight to 10 days with Kodak XAR film and 3 Dupont Quanta III screens at -70 C.

Preparation of RNA.



Bone marrow aspirate RNA: 30 cc of normal bone marrow was aspirated from a single site from the posterior iliac crest of a normal volunteer with a Jamshidi needle and standard sterile procedures. The aspirate was immediately placed on ice, and after clotting, 7 cc of serum was removed and the remainder of the sample was transferred rapidly to 50 cc sterile tubes with 1.5 volumes of 4 M Guanidine isothiocyanate solution (4 M guanidine isothiocyanate, 0.5% N-lauroylsarcosine, 25 mM sodium citrate, 0.1 M 2-mercaptoethanol). Samples were immediately homogenized with a NaOH cleaned high speed (Polytron) tissue homogenizer, and RNA prepared using a modified Chirgwin procedure (Chirgwin *et al.*, 1979). The 360  $\mu$ g total RNA sample was stored in ethanol at -20 C until Poly A selected.

Poly A selection: 200  $\mu$ g of bone marrow aspirate total RNA was poly A selected according to a method by Cho (Wahli and Martinex, 1991). The integrity of the 9  $\mu$ g of poly A selected RNA obtained was confirmed by gel electrophoresis.

Fractionated peripheral blood cell total and poly A RNA isolation: A leukapheresis pack was obtained from the American Red Cross. Monocytes, lymphocytes and neutrophils were separated by standard procedures (Kitano *et al.*, 1991) with the monocytes and lymphocytes separated by overnight adherence. The neutrophil pellet yielded 106  $\mu$ g of poly A RNA using the procedure described above. Monocytes separated by standard procedures (Kitano *et al.*, 1991) were scraped gently off plates and pelleted, yielding 13  $\mu$ g total RNA by the above procedure. Monocytes were isolated from three leukapheresis packs by Ficoll-Paque (Pharmacia) separation (Kitano *et al.*, 1991), followed by overnight adherence in Iscove's modified Dulbecco's media (Gibco) with 15% fetal bovine serum (Gemini) and 5% heat inactivated human AB serum at 37 C. Cells were lysed *in situ* on the plates with 4 M Guanidine. 5  $\mu$ g poly A RNA was prepared from the total RNA

using the Fastrack kit (Invitrogen). Lymphocyte total RNA and poly A selected RNA were obtained from Ficoll-Paque (Pharmacia) separated fractions using the same procedures described above.

Cell line RNA: The following human hematopoietic cell lines were used for RNA and DNA preparation: T cell: CCRF-HSB-2, CCRF-CEM, MOLT-4, JURKAT B Cell:-Raji, NOR 25 (an EBV transformed normal adult peripheral blood B cell line kindly provided by Nancy Perillo) murine pre B: 18.81, Wehi 231,70Z3, (kindly provided by O. Witte) Myeloid: HL60, KG-1, K562, THP-1.

All cells were cultured in Iscove's modified Dulbecco's medium (Gibco) with 10% fetal bovine serum (Gemini), 100  $\mu$ /ml penicillin G, 0.1 mg/ml streptomycin (Sigma, L-glutamine 0.4 mM (Gibco). The NOR-25, THP-1, 18.81, Wehi 231, and 70Z3 cell lines had  $5 \times 10^{-5}$  M  $\beta$  mercaptoethanol (Gibco) added. NOR-25 also required 0.01 M Hepes (Gibco). A quantity of 20-40  $\mu$ g of Poly A selected RNA was made from  $1 \times 10^8$  cells using the Fastrack kit (Invitrogen).

#### Sequence of human PPAR- $\gamma$ clones.

Two PPAR- $\gamma$  clones were identified from the degenerate oligonucleotide screen of the normal human bone marrow cDNA library. Both share a significant percentage of amino acid identity with the PPAR family of gene products, and especially with xenopus PPAR- $\gamma$  (see below). These two clones, one full-length (clone 14) and one incomplete (clone 22) are therefore identified as human versions of the PPAR- $\gamma$  gene.

The full length PPAR- $\gamma$  cDNA (clone 14), was isolated and sequenced from both ends. This clone was 1844 bp long, with the open reading frame beginning at bp 179, and the stop codon at bp 1604. The 1428 BP open reading frame encodes a 476 amino acid nuclear receptor

protein, with a molecular weight of approximately 54.2 kDa. The beginning of the A/B region contains the amino acid sequence MVDT, as has been reported for other members of this family. Clone 14 contains all functional components characteristic of nuclear receptors, and shows similarities and conservative base changes with the human NUC 1 receptor, the murine and rat PPAR- $\alpha$ , and the xenopus PPAR  $\alpha$ ,  $\beta$  and  $\gamma$  receptors. Clone 14 shows the greatest similarity (73% overall amino acid identify) to the xenopus PPAR- $\gamma$  cDNA (FIG. 1).

Sequencing of the second clone (clone 22) revealed an artifactual splice at a Hind III site (bp 597) in the C region, causing abrupt loss of 9 highly conserved C region amino acids. The next 1247 bp's are identical to clone 14, confirming the majority of the sequence obtained from the full length receptor contained in clone 14.

**Example 2: mRNA Expression Studies.**

Isolation of bone marrow stromal cells: Bone marrow stromal cells were obtained from a normal donor bone marrow harvest, filtered through a 200 micron wire mesh screen, washed with saline and suspended in Dexter's media, (cells kindly donated by J. Nolte and D. Kohn, M.D., L.A. Children's Hospital). Cells were maintained in DOM (IMDM, 15% horse serum, 15% fetal calf serum,  $5 \times 10^{-5}$  M  $\beta$  mercaptoethanol,  $10^{-3}$  M hydrocortisone, with pen/strep) for 2 additional passages (total of 4 passages) before harvesting. Collagenase was used during passaging of the cells to allow cells to retain their capability to differentiate towards adipose as well as fibroblastic cell types (Nolte *et al.*, 1992). Poly A mRNA was obtained from  $1 \times 10^8$  cells using the Fastrack kit.

Patient samples: Patients seen at UCLA with acute leukemia or chronic myelogenous leukemia provided informed consent to donate blood, as approved by the UCLA Human Subject Protection Committee. Fifteen to

30 ml of peripheral blood was obtained from patients with active leukemia and the mononuclear cell fraction was isolated by Ficoll-Hypaque density separation. Total RNA was prepared according to a modification of the Chirgwin method (Chirgwin *et al.*, 1979).

5            Fetal RNA: Tissue samples from fetuses with no known genetic abnormality or chemical exposure were provided by elective surgical abortions performed between 11-24 weeks postconception, after obtaining the patient's informed consent. Samples were obtained within minutes of pregnancy termination and were frozen immediately in liquid nitrogen and  
10           stored at -80 C. The conceptional age of abortuses was determined by measurement of fetal foot length (Moore, 1982). Prior to freezing, samples of fetal cortex were dissected away from the central diencephalon and the basal ganglia, and were freed of membrane and choroid plexus; the meninges were removed from spinal cords. Samples of placental tissue were obtained  
15           from the fetal aspect following removal of the amnion and chorion.

RNA was prepared from fetal cortex, cerebrum, pooled intact and partial spinal cords, kidney, lung, placenta and liver. Total RNA was prepared from frozen tissue fragments by homogenization in GITC using the methods of Chirgwin et al (Chirgwin *et al.*, 1979) or Chomczynski and  
20           Sacchi (Chomczynski and Sacchi, 1987). An RNA fraction enriched in poly A+ RNA was isolated from total RNA by affinity chromatography (Avid and Leder, 1972). Preparation of an additional northern blot containing fetal liver RNA was previously described (Kronquist *et al.*, 1990).

25           Northern blots: Formaldehyde 0.66 M to 2.0 M, agarose 1%, 1x MOPs gels were loaded with either methyl mercury or heat denatured total cellular or poly A selected RNA. Gels were transferred to either Hybond (Amersham) or Nytran (Schleicher and Schuell) membranes using standard platform transfer techniques in 10x or 20x SSC.

Northern blot analysis was performed to examine the pattern of expression of this transcription factor in hematopoietic and bone marrow stromal cells. Expression of two different PPAR- $\gamma$  mRNA transcripts was observed in hematopoietic cells. Two PPAR- $\gamma$  transcripts of similar size are also present in the xenopus system [12]. The human 1.85 kb transcript is large enough to contain all the functional regions present in the 1428 base pair open reading frame cDNA sequence cloned from the bone marrow library (*see* FIG. 2). The 0.65 kb transcript by size alone cannot contain all the functional regions of a nuclear receptor (e.g. the ligand binding domain is 678 bp, the DNA binding domain is 196 bp and a short linker region would be 220-234 bp for a total 1.1 kb). The partial clone (clone 22) was not considered to represent the short 650-700 bp transcript based on length, since it contained 1247 bps with 1010 bps identical to clone 14 full length receptor open reading frame.

The larger transcript (PPAR- $\gamma$  1) is expressed in a variety of malignant hematopoietic cell lines, normal peripheral blood monocytes cultured overnight, and primary cultured stromal cells. (*See* Table 1.) The shorter 0.65 kb transcript (PPAR- $\gamma$  2) is the only form seen in circulating peripheral blood neutrophil and lymphocyte fractions, as well as in circulating mononuclear cells from patients with acute lymphocytic leukemia and acute and chronic myeloid leukemia. No PPAR- $\gamma$  mRNA was detected in Jurkat, HSB, Raji, K562, or KG-1 cell lines.

Expression of only the smaller transcript was detected in the peripheral blood of adult patients with acute myeloid leukemia (AML), chronic myeloid leukemia (CML), CML in blast crisis and acute lymphocytic leukemia (ALL). Not all patient samples showed expression of PPAR- $\gamma$ . Blast counts varied somewhat, and it was considered that some of the signal could have been generated by the few normal lymphocytes or monocytes remaining after a Ficoll-hypaque separation. However, 20  $\mu$ g total RNA

from normal peripheral blood lymphocytes shows no signal under the same hybridization conditions.

5 A 5- $\mu$ g poly A selected RNA was obtained from peripheral blood monocytes separated from lymphocytes by overnight adherence in Iscove's media supplemented with 15% fetal calf serum. The dominant signal is the long PPAR- $\gamma$  1 transcript. The short transcript signal may be from contaminating lymphocytes, a subpopulation of monocytes such as activated monocytes or macrophages, or coexpressed in the same cells as the long transcript.

10 Primary bone marrow stromal cells cultured for a total of 4 passages expressed only the 1.8 kb (PPAR- $\gamma$  1) transcript. In contrast to isolated hematopoietic cell populations, other human organ systems show expression of both transcripts, (colon, bladder, kidney, skeletal muscle, liver, spleen, placenta, and stomach) with the 1.85 kb mRNA being dominant in colon, muscle, placenta and bladder. Both transcripts were also seen in fetal organs (16-19 gestational weeks) with varying ratios of each transcript in fetal cerebral cortex, lung (very faint) and kidney. The 1.85 kb transcript was dominant in fetal kidney, and the 0.67 transcript was dominant in fetal liver and lung.

20 Normal peripheral blood lymphocytes, polymorphonuclear leukocytes, and circulating leukemia cells from patients with ALL, CML, and CML in blast crisis, express only the shorter 0.65 kb PPAR- $\gamma$  transcript. In contrast, hematopoietic leukemia cell lines derived from acute myeloid leukemia (HL60 - M3, U937 and THP-1, monocytic/histiocytic with FC receptors and phagocytic capabilities) express only the 1.85 kb mRNA, as detected by  
25 northern blot analysis. Cultured stromal cells also only express the 1.85 kb transcript, indicating that some aspect of culture conditions, such as rapid cell division, and/or increased utilization of fatty acid  $\beta$  or  $\omega$  oxidation

pathways may be reflected in PPAR- $\gamma$  transcription. Another consideration is that the ligand for PPAR is likely to be a product of eicosanoid metabolism (Gottlicher *et al.*, 1992; Muerhoff *et al.*, 1992). Many types of eicosanoid metabolism in the peripheral blood compartment require the concurrent presence of more than one cell type (Marcus, 1990). Platelets, neutrophils, and erythrocytes may participate in this eicosanoid transcellular metabolism. For example, 5-HETE is produced by neutrophils from platelet derived precursors. This can account for the fact that an eicosanoid metabolite binding transcription factor regulating a key enzyme of eicosanoid  $\omega$  oxidation is transcribed differently in the peripheral blood compartment than it is in tissue culture. Other differences involve growth factors. Overnight culture of human leukemia cells induce high levels of IL-1, which can induce the *in vitro* expression of the myeloid growth factor GM-CSF [Kaufman, 1988 #57]. The peripheral blood monocyte fraction which also expressed predominately the 1.85 kb transcript was exposed to Iscove's media with 15% fetal bovine serum overnight during separation by adherence. All cultured cell lines were exposed to 10% fetal calf serum. In contrast, the leukemic cells fresh from patient peripheral blood, with manipulations other than ficoll separation, show expression of only the short transcript.

In examining the lymphoid lineage, it is apparent that peripheral blood lymphocytes (95% T) and ALL circulating blasts express short PPAR- $\gamma$  2 transcript, while neither of the two T lymphoid cells lines studied (HSB and Jurkat) expressed any PPAR transcript. Normal peripheral blood B cells transformed with Epstein Barr virus in culture expressed the long transcript. In acute myeloid leukemia, cell lines (HL60, THP-1, U937,) expressed the long PPAR- $\gamma$  1 transcript, and some fresh acute myeloid leukemic cells produced the short PPAR- $\gamma$  2 transcript.

The main purpose of examining the leukemic patient peripheral blood samples was to ascertain if PPAR- $\gamma$  expression was restricted to any diagnostic category. PPAR- $\gamma$  expression of the short transcript was seen in ALL, CML, and AML. Whether the presence or absence of PPAR- $\gamma$  expression correlates with any clinical parameter is difficult to determine with currently available data.

PPAR- $\gamma$  has novel regulatory aspects and appears to be widely expressed in the human hematopoietic system, and in a variety of both adult and fetal organs. The ratio of the two transcripts, 1.85 kb (PPAR- $\gamma$  1) and 0.65 kb (PPAR- $\gamma$  2) vary from organ to organ, and a detailed study of the hematopoietic system suggests that different populations of cells in an organ system may express one transcript or the other preferentially.

Previous studies of PPARs have focused on the expression of all three PPARs in the xenopus system (Dreyer *et al.*, 1992), existence of PPAR alpha in humans (Sher *et al.*, 1993) and rats (Gottlicher *et al.*, 1992), and expression of PPAR alpha in murine systems [Beck, 1992 #25; Issemann *et al.*, 1990; Tugwood *et al.*, 1992). XPPAR- $\gamma$  expression is seen in xenopus oocytes, liver, kidney and abdominal body fat. It appears the hPPAR- $\gamma$  is expressed in a great variety of human organ systems.



TABLE 1

LINEAGE	NAME	PPAR $\gamma$ 1 (1.85 kb)	PPAR $\gamma$ 2 (0.65 kb)	RAR a (3.6/2.4 kb)
<u>Cultured Malignant Hematopoietic Cell Lines</u>				
Lymphoid				
T-Lymphoblastoid	HSB-2	-	-	+++
	MOLT-4F	-	-	+++
	CCRF-CEM	-	-	+++
	JURKAT	-	-	+++
	RAJ	-	-	+++
	NOR-25	+++	-	+++
Myeloid				
Promyelocytic	HL60	+++	-	+++
Myeloid-monocytic	U937	+++	-	+++
(histiocytic, FC	THP-1	+++	-	+++
receptors, phago-	KG-1	-	-	+++
cytic, AML)				
Erythroid				
Myeloid-Erythroid	K562	-	-	+++
heme + expression				
<u>Peripheral Blood, Bone Marrow Compartments</u>				
Peripheral blood,	PMNs	-	+++	+++++++
(fractionated, normal)	Lymphocytes (95% T)	-	+++	+++
	Monocytes (adherence	+	++	+++
	purified, in culture			
	overnight			
Total bone marrow aspirate		+++	+	+++
stroma/hematopoietic cells				
Cultured primary bone marrow		+++	-	+++
cells, fibroblastic and				
adipocytic differentiation				
Peripheral Bld/Leukemic Pts.				
AML				
	M1	0/2-	0/2-	ND
	M2	0/4-	2/4+	ND
	M3	ND	ND	ND
	M4	0/2-	1/2+	ND
	M5	0/1-	1/1+	ND
	Unspecified	0/2-	1/2+	ND
		0/8-	7/8+	ND
		0/5-	1/5+	ND
	L1	0/1-	1/1+	ND
CML		0/4-	2/4+	ND
CML in blast crisis		0/2-	2/2+	ND
ALL		0/1+	2/1+	ND
	L2			ND
	bi-phenotypic			ND
	NK cell, TdT-			ND

**Example 3: Genomic alterations.**

**Southern blot analysis:** Genomic DNA was isolated from peripheral blood leukocytes of 10 normal volunteers, from 13 cell lines, and normal primary cultured bone marrow stromal cells (Gatti *et al.*, 1989; McBride *et al.*, 1989). 5 mg of cell line and stromal cell DNA was digested to completion with EcoR I, Bam HI, and Hind III restriction enzymes for the cell line southern under conditions specified by the supplier. DNA from the normal volunteers was digested with restriction endonucleases EcoR I, Bam HI, Hind III, Xba I, Sac I, Taq I, Pvu II, Pst I, Bgl II, Msp I, EcoR V, and Kpn I. DNA fragments were fractionated by 0.7 or 0.8% agarose gel electrophoresis, and transferred to positively charged nylon membranes in 0.5 N NaOH as described. A 1.6 kb full length hPPAR- $\gamma$ , and a plasmid control probe were used for cell line southern blot analyses. A 0.8 kb A,B,C, and D region hPPAR- $\gamma$  probe was used for hybridization to normal volunteer DNA for Southern blot RFLP analyses. Probes were labelled as described above. The membranes were hybridized (at 42° C in 50% formamide) and washed (in 0.1X SSC at 55-58° C) at high stringency allowing less than 10% sequence divergence as described (McBride *et al.*, 1989).

**Somatic Cell Hybrids:** The isolation and characterization of a panel of human-rodent somatic cell hybrids retaining subsets of human chromosomes has been described previously (McBride *et al.*, 1982). Hybrid cell lines were characterized for the presence of all human chromosomes except Y by standard isoenzyme analyses, by Southern analysis with probes from previously localized genes, and frequently, by cytogenetic analysis.

**Linkage analysis:** Southern blots of Xba I digests of DNAs from 40 large, three-generation CEPH families (Dausset *et al.*, 1990) were used for hybridization with a 0.8 kb A/B, C and D region hPPAR- $\gamma$  cDNA probe. RFLP typing was performed by standard Southern blot analysis under high stringency conditions. All parental DNAs were initially examined, and all

family members from informative matings (i.e., those with one or both heterozygous parents) were then examined. Two-point and multipoint linkage analysis of PPAR- $\gamma$  versus loci typed in the CEPH database version 5 were performed using LINKAGE version 5.1 for microcomputers (Lathrop *et al.*, 1984). An infrequent Taq I polymorphism (B1:B2 = 7.1:5.3kb) detected in one CEPH parent was used to genotype CEPH family 1413 and the results with Xba I and Taq I were combined as a haplotype in this family.

Chromosomal Localization of hPPAR- $\gamma$ : The gene was localized by Southern blot analysis of a panel of EcoRI-digested human-rodent somatic cell hybrid DNAs with a hPPAR- $\gamma$  cDNA probe. The three hybridizing bands in human DNAs were readily distinguished from cross-hybridizing bands in rodent DNAs (not shown). All human bands co-segregated and the gene could be unambiguously assigned to human chromosome 3 and it segregated discordantly (> 18%) with all other human chromosomes. The gene was further localized on the short arm of chromosome 3 by examination of two hybrids containing spontaneous breaks or deletions involving this chromosome. One hybrid contained a large deletion in the short arm with loss of distal 3p markers RAF1 (3p25), THRB (3p24.1-p22), and ACY1 (3p21), but retention of DNF15S2 (3p21.2-21.3), CCHL1A2 (3p14.3) (Chin *et al.*, 1991) as well as two other more centromeric 3p markers and all loci on 3q. The other hybrid contained a break in proximal 3q with retention of long arm markers and loss of all short arm markers. The hPPAR- $\gamma$  gene was absent from both of these hybrids indicating that it must be located in the region 3p21-p25.

The gene could be further localized to band 3p25 by genetic linkage analysis after identification of a high frequency RFLP at the hPPAR- $\gamma$  locus (PPARG). The 0.8 kb hPPAR- $\gamma$  cDNA probe was used for blot hybridization with genomic DNAs, and an RFLP was detected in Xba I

digests with this probe. Allelic bands of 4.0 kb (A2) and 9.0 kb (A1) were found in addition to constant bands of 3.0, 4.4, and 7.2 kb lengths. In 80 CEPH parents, the allele frequencies were: A1:A2 = 0.5:0.5. The Xba I polymorphism was used for linkage analysis in the CEPH families. One or both parents was a heterozygote in 34 of the 40 total families, and both parents were heterozygotes in 18 of these families. All family members were examined in the informative families and the genotypes were entered into the CEPH database and used for linkage analysis with other published markers from chromosome 3p. Several errors were detected and corrected.

Multipoint linkage analysis of PPARG with other published markers (Tory *et al.*, 1992) in the 3p26-p22 region was used to order PPARG with respect to these other loci. The results do not permit ordering of PPAR- $\gamma$  and RAF1, but both loci are centromeric to D3S18 and telomeric to D3S588 and THRB, within band 3p25.

Genomic alterations: Southern blot analysis was performed to investigate PPAR- $\gamma$  gene integrity in those cell lines that did not express PPAR- $\gamma$ . Two of the five cell lines which did not express PPAR- $\gamma$  transcripts (Raji and Jurkat) had major genomic alterations. The increase in intensity of two of the bands seen on the Eco R1, Bam H1, and Hind III digests of Raji cell genomic DNA, relative to the respective germline bands, is suggestive of amplification of an abnormal gene arrangement. The deletions seen in the Jurkat cells are consistent with abnormalities (deletions) of 3p previously identified in these cells[50] consistent with the localization of the hPPAR- $\gamma$  gene. The other three cell lines (HSB, K562, and KG-1) showed normal germline patterns. Polymorphism studies in CEPH families and other southern blot studies demonstrated that the patterns seen in Jurkat and Raji are not consistent with natural polymorphisms.



## REFERENCES CITED

The references listed below as well as all references cited in the specification are incorporated herein by reference to the extent that they supplement, explain, provide a background for or teach methodology, techniques and/or compositions employed herein.

5

Adelman *et al.*, *DNA* 2:183 (1983).

Agura, *et al.*, *Blood*, **79**:602-609 (1992).

*Antibodies "A Laboratory Manual*, E. Howell and D. Lane, Cold Spring Harbor Aronica Laboratory, (1988).

10

Avid *et al.*, *Proc. Natl. Acad. Sci USA*, **69**:1408-1412 (1972).

Beck *et al.*, *Proc. R. Soc. London B* **247**: 83-87 (1992).

Beier *et al.*, *European Journal of Cell Biology* **46**:383-393 (1988).

Berioli *et al.*, *Cardiologia* **35**: 335-340 (1990).

Bertin *et al.*, *J. Biol. Chem.* **267**(12):8200 (1992).

15

Blaauboer *et al.*, *Biochemical Pharmacology*, **40**:521-528 (1990).

Blumberg *et al.*, *Natl. Acad. Sci. USA*, **89**:2321-2325 (1992).

Bolivar *et al.*, *Gene*, **2**:95 (1977).

Boshart *et al.*, *Cell* **41**:521 (1985).

Bouvier *et al.*, *Mol. Pharmacol.* **33**:133 (1988).

20

Cattley *et al.*, *Cancer Res.* **49**: 3246-3251 (1989).

Chang *et al.*, *Nature*, **375**:615 (1978).

Chin *et al.*, *Genomics* **11**:914-919 (1991).

Chirgwin *et al.*, *Biochemistry*, **18**:5294-5299 (1979).

Chomczynski *et al.*, *Anal. Biochemisrty*, **162**:156-159 (1987).

25

Crea *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **75**:5765 (1978).

Danboldt *et al.*, *Biochemistry* **29**(28):6734 (1990).

Danielian *et al.*, *EMBO*, **11**:1025-1033 (1992).

Dausset *et al.*, *Genomics* **6**:575-577 (1990).

Debois *et al.*, *Cell* **67**:731-740 (1991).

- Devereaux *et al.*, *Nucleic Acids Research*, **12**:387-395 (1984).
- Dreyer *et al.*, *Cell*, **68**:879-887 (1992).
- Eacho *et al.*, *Toxicol. Appl. Pharmacol.* **83**: 430-437 (1986).
- Elder *et al.*, *J. Invest. Derm.* **96**: 425-433 (1991).
- 5 Evans, *Science*, **240**:889-895 (1988).
- Feng *et al.*, *J. Mol Evolution*, **25**:351-36 (1987).
- Ferruti *et al.*, *Crit. Rev. Ther. Drug Carrier Syst.* **2**:117-136 (1986).
- Fiers *et al.*, *Nature* **273**:113 (1978).
- Forman *et al.*, *Mol. Endocrinol.* **4**:1293-1301 (1990).
- 10 Gabizon *et al.*, *Cancer Res.* **50**:6371-6378 (1990).
- Gatti *et al.*, *Disease Markers* **7**:105-112 (1989).
- Giguere *et al.*, *Nature* **330**:624-629 (1987).
- Goeddel *et al.*, *Nucleic Acids Res.*, **8**:4057 (1980).
- Goeddel *et al.*, *Nature*, **281**:544 (1979).
- 15 Gottlicher *et al.*, *Proc. Natl. Acad. Sci USA*, **89**:4653-4657 (1992).
- Hess *et al.*, *J. Adv. Enzyme Reg.* **7**:149 (1968).
- Hitzeman *et al.*, *J. Biol. Chem.* **255**:2073 (1980).
- Holland *et al.*, *Biochemistry* **17**:4900 (1978).
- Howell *et al.*, *Blood*, **75**:721-729 (1990).
- 20 Issemann *et al.*, *Nature*, **347**:645-650 (1990).
- Itakura *et al.*, *Science*, **198**:1056 (1977).
- Jones, *Genetics* **85**:12 (1977).
- Kasai *et al.*, *Cancer Res.* **49**: 2603-2605 (1989).
- King *et al.*, *Science* **250**:121 (1990).
- 25 Kingsman *et al.*, *Gene* **7**:141 (1979).
- Kitano *et al.*, *Blood*, **77**:1625-1626 (1991).
- Kliwer *et al.*, *Nature* **358**: 771-774 (1992).
- Klug *et al.*, *Trends in Biochem. Sci.* **12**, 468 (1987).
- Kronquist *et al.*, *Prenatal Diagnosis*, **10**:739-751 (1990).
- 30 Kruse *et al.*, *Tissue Culture*, Academic Press (1973).
- Kyte *et al.*, *J. Mol. Biol.*, **157**:105-132, (1982).

- Lathrop *et al.*, *Proc. Natl. Acad. Sci. USA*. **81**:3443-3446 (1984).
- Laudet *et al.*, *EMBO*, **11**:1003-1013 (1992).
- Marcus, *Academic Press*, 585-599 (1990).
- Marsman *et al.*, *Cancer Res.* **48**: 6739-6744 (1988).
- 5 McBride *et al.*, *J. Exp. Med.* **155**:1480-1490 (1982).
- McBride *et al.*, *Genomics*. **5**:561-573 (1989).
- Menger *et al.*, *Blood*, **72**:567-572 (1988).
- Messing *et al.*, Third Cleveland Symposium on Macromolecules and  
Recombinant DNA, Editor A. Walton, Elsevier, Amsterdam (1981).
- 10 Moore, Development of the Human, (1982).
- Muerhoff *et al.*, *The Journal of Biological Chemistry*, **267**:19051-19053  
(1992).
- Nolta *et al.*, *J. Clinical Investigation* , **90**:342-348 (1992).
- O'Malley *et al.*, *Mol. Endocrin.* **6**: 1359-1361 (1992).
- 15 O'Malley *et al.*, *Mol. Endocrin.* **6**: 1359-1361 (1992).
- Okayama *et al.*, *Mol. Cell Biol.* **3**:280 (1983).
- Petkovich *et al.*, *Nature* **330**:444-450 (1987).
- Ranade, *J. Clin. Pharmacol.* **29**:685-694 (1989).
- Reddy *et al.*, *CRC Crit. Rev. Toxicol.* **12**: 1-58 (1983).
- 20 Rowley, *J. Clin. Oncol.* **6**:194 (1988).
- Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring  
Harbor Laboratory Press, Cold Spring Harbor, NY) (1989).
- Schmidt *et al.*, *Molecular Endocrinology* **6**:1634-1641 (1992).
- Schule *et al.*, *Proc. Natl. Acad. Sci. USA* **88**:6092-6096 (1991).
- 25 Seeburg, *DNA* **1**:239 (1982).
- Sharif *et al.*, *Cell*, **66**:885-893 (1991).
- Sher *et al.*, *Biochemistry* **32**:5598-5604 (1993).
- Short *et al.*, *Nucleic Acids Research*, **16**:7583-7600 (1988).
- Siebenlist *et al.*, *Cell*, **20**:269 (1980).
- 30 Snow and Judd, *Experimental Cell Research*, **171**:389-403 (1987).
- Staden, *Nucleic Acids Research*, **14**:217-231 (1986).



Stinchcomb *et al.*, *Nature*, **282**:39 (1979).

Sun, Hematology, an Atlas and Diagnostic Guide, 1 ed, W. B. Saunders, Philadelphia, PA (1983).

Tabor and Richardson, *Proc. Natl. Acad. Sci USA*, **86**:4076-4080 (1989).

5 Thomsen *et al.*, *Proc. Natl. Acad. Sci USA* **81**:659 (1984).

Tory *et al.*, *Genomics* **13**:275-286 (1992).

Truss *et al.*, *Endocrine Rev.* **14**: 459-479 (1993).

Tschemper *et al.*, *Gene* **10**:157 (1980).

Tsukiyama *et al.*, *Mol. Cell Biol.* **12**: 1286-1291 (1992).

10 Tugwood *et al.*, *EMBO* **11**: 433-439 (1992).

Umesono *et al.*, *Cell*, **65**:1255-1266 (1991).

Wahli *et al.*, *FASEB*, **5**:2243-2249 (1991).

Warren *et al.*, *Cancer Res.* **40**: 36-41 (1980).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Greene, Marianne E.  
Blumberg, Bruce

(ii) TITLE OF INVENTION: Human Peroxisome Proliferator  
Activated Receptor Gamma:  
Compositions and Methods

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Arnold, White & Durkee  
(B) STREET: 321 North Clark Street, Suite 800  
(C) CITY: Chicago  
(D) STATE: IL  
(E) COUNTRY: USA  
(F) ZIP: 60610

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US UNKNOWN  
(B) FILING DATE: 08-OCT-1993  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Coughlin, Daniel F.  
(B) REGISTRATION NUMBER: 36,111  
(C) REFERENCE/DOCKET NUMBER: ARCD098

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 312-744-0090  
(B) TELEFAX: 312-755-4489

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1844 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 179..1603

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGACCTTAC CCCAGGCGGC CTTGACGTTG GTCTTGTCGG CAGGAGACAG CACCATGGTG	60
GGTTCTCTCT GAGTCTGGGA ATTCCCGAGC CCGAGCCGCA GCCGCCGCTT GGGGGGCTTG	120
GTGCGGCTC GAGGACACCG GAGAGGGGCG CCACGCCGCC GTGGCCGCAG AAATGACC	178

	ATG GTT GAC ACA GAG ATC GCA TTC TGG CCC ACC AAC TTT GGG ATC AGC Met Val Asp Thr Glu Ile Ala Phe Trp Pro Thr Asn Phe Gly Ile Ser 1 5 10 15	226
5	TCC GTG GAT CTC TCC GTA ATG GAA GAC CAC TCC CAC TCC TTT GAT ATC Ser Val Asp Leu Ser Val Met Glu Asp His Ser His Ser Phe Asp Ile 20 25 30	274
	AAG CCC TTC ACT ACT GTT GAC TTC TCC AGC ATT TCT ACT CCA CAT TAC Lys Pro Phe Thr Thr Val Asp Phe Ser Ser Ile Ser Thr Pro His Tyr 35 40 45	322
10	GAA GAC ATT CCA TTC ACA AGA ACA GAT CCA GTG GTT GCA GAT TAC AAG Glu Asp Ile Pro Phe Thr Arg Thr Asp Pro Val Ala Asp Tyr Lys 50 55 60	370
15	TAT GAC CTG AAA CTT CAA GAG TAC CAA AGT GCA ATC AAA GTG GAG CCT Tyr Asp Leu Lys Leu Gln Glu Tyr Gln Ser Ala Ile Lys Val Glu Pro 65 70 75 80	418
	GCA TCT CCA CCT TAT TAT TCT GAG AAG ACT CAG CTC TAC AAT AAG CCT Ala Ser Pro Pro Tyr Tyr Ser Glu Lys Thr Gln Leu Tyr Asn Lys Pro 85 90 95	466
20	CAT GAA GAG CCT TCC AAC TCC CTC ATG GCA ATT GAA TGT CGT GTC TGT His Glu Glu Pro Ser Asn Ser Leu Met Ala Ile Glu Cys Arg Val Cys 100 105 110	514
	GGA GAT AAA GCT TCT GGA TTT CAC TAT GGA GTT CAT GCT TGT GAA GGA Gly Asp Lys Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Glu Gly 115 120 125	562
25	TGC AAG GGT TTC TTC CGG AGA ACA ATC AGA TTG AAG CTT ATC TAT GAC Cys Lys Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys Leu Ile Tyr Asp 130 135 140	610
30	AGA TGT GAT CTT AAC TGT CGG ATC CAC AAA AAA AGT AGA AAT AAA TGT Arg Cys Asp Leu Asn Cys Arg Ile His Lys Lys Ser Arg Asn Lys Cys 145 150 155 160	658
	CAG TAC TGT CGG TTT CAG AAA TGC CTT GCA GTG GGG ATG TCT CAT AAT Gln Tyr Cys Arg Phe Gln Lys Cys Leu Ala Val Gly Met Ser His Asn 165 170 175	706
35	GCC ATC AGG TTT GGG CGG ATC GCA CAG GCC GAG AAG GAG CTG TTG Ala Ile Arg Phe Gly Arg Ile Ala Gln Ala Glu Lys Glu Lys Leu Leu 180 185 190	754
	GCG GAG ATC TCC AGT GAT ATC GAC CAG CTG AAT CCA GAG TCC GCT GAC Ala Glu Ile Ser Ser Asp Ile Asp Gln Leu Asn Pro Glu Ser Ala Asp 195 200 205	802
40	CTC CGT CAG GCC CTG GCA AAA CAT TTG TAT GAC TCA TAC ATA AAG TCC Leu Arg Gln Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile Lys Ser 210 215 220	850
45	TTG CCG CTG ACC AAA GCA AAG GCG AGG GCG ATC TTG ACA GGA AAG ACA Phe Pro Leu Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly Lys Thr 225 230 235 240	898
	ACA GAC AAA TCA CCA TTC GTT ATC TAT GAC ATG AAT TCC TTA ATG ATG Thr Asp Lys Ser Pro Phe Val Ile Tyr Asp Met Asn Ser Leu Met Met 245 250 255	946
50	GGA GAA GAT AAA ATC AAG TTC AAA CAC ATC ACC CCC CTG CAG GAG CAG Gly Glu Asp Lys Ile Lys Phe Lys His Ile Thr Pro Leu Gln Glu Gln 260 265 270	994
	AGC AAA GAG GTG GCC ATC CGC ATC TTT CAG GGC TGC CAG TTT CGC TCC Ser Lys Glu Val Ala Ile Arg Ile Phe Gln Gly Cys Gln Phe Arg Ser 275 280 285	1042

	GTG GAG GCT GTG CAG GAG ATC ACA GAG TAT GCC AAA AGC ATT CCT GGT Val Glu Ala Val Gln Glu Ile Thr Glu Tyr Ala Lys Ser Ile Pro Gly 290 295 300	1090
5	TTT GTA AAT CTT GAC TTG AAC GAC CAA GTA ACT CTC CTC AAA TAT GGA Phe Val Asn Leu Asp Leu Asn Asp Gln Val Thr Leu Leu Lys Tyr Gly 305 310 315 320	1138
	GTC CAC GAG ATC ATT TAC ACA ATG CTG GCC TCC TTG ATG AAT AAA GAT Val His Glu Ile Ile Tyr Thr Met Leu Ala Ser Leu Met Asn Lys Asp 325 330 335	1186
10	GGG GTT CTC ATA TCC GAG GGC CAA GGC TTC ATG ACA AGG GAG TTT CTA Gly Val Leu Ile Ser Glu Gly Gln Gly Phe Met Thr Arg Glu Phe Leu 340 345 350	1234
15	AAG AGC CTG CGA AAG CCT TTT GGT GAC TTT ATG GAG CCC AAG TTT GAG Lys Ser Leu Arg Lys Pro Phe Gly Asp Phe Met Glu Pro Lys Phe Glu 355 360 365	1282
	TTT GCT GTG AAG TTC AAT GCA CTG GAA TTA GAT GAC AGC GAC TTG GCA Phe Ala Val Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala 370 375 380	1330
20	ATA TTT ATT GCT GTC ATT ATT CTC AGT GGA GAC CGC CCA GGT TTG CTG Ile Phe Ile Ala Val Ile Ile Leu Ser Gly Asp Arg Pro Gly Leu Leu 385 390 395 400	1378
	AAT GTG AAG CCC ATT GAA GAC ATT CAA GAC AAC CTG CTA CAA GCC CTG Asn Val Lys Pro Ile Glu Asp Ile Gln Asp Asn Leu Leu Gln Ala Leu 405 410 415	1426
25	GAG CTC CAG CTG AAG CTG AAC CAC CCT GAG TCC TCA CAG CTG TTT GCC Glu Leu Gln Leu Lys Leu Asn His Pro Glu Ser Ser Gln Leu Phe Ala 420 425 430	1474
30	AAG CTG CTC CAG AAA ATG ACA GAC CTC AGA CAG ATT GTC ACG GAA CAC Lys Leu Leu Gln Lys Met Thr Asp Leu Arg Gln Ile Val Thr Glu His 435 440 445	1522
	GTG CAG CTA CTG CAG GTG ATC AAG AAG ACG GAG ACA GAC ATG AGT CTT Val Gln Leu Leu Gln Val Ile Lys Lys Thr Glu Thr Asp Met Ser Leu 450 455 460	1570
35	CAC CCG CTC CTG CAG GAG ATC TAC AAG GAC TTG TAC TAGCAGAGAG His Pro Leu Leu Gln Glu Ile Tyr Lys Asp Leu Tyr 465 470 475	1616
	TCCTGAGCCA CTGCCAACAT TTCCCTTCTT CCATTTCAC TATTCTGAGG GAAAACTGA	1676
	CCATAAGAAA TTTACTGTGA AAAAGCGTTT TAAAAAGAAA AGGGTTTAGA ATATGATCTA	1736
	TTTTATGCAT ATTGTTTATA AAGACACATT TACAATTTAC TTTTAATATT AAAAATTACC	1796
40	ATATTATGAA GTTGCAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA	1844

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 476 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Val Asp Thr Glu Ile Ala Phe Trp Pro Thr Asn Phe Gly Ile Ser  
1 5 10 15

Ser Val Asp Leu Ser Val Met Glu Asp His Ser His Ser Phe Asp Ile  
 20 25 30  
 Lys Pro Phe Thr Thr Val Asp Phe Ser Ser Ile Ser Thr Pro His Tyr  
 35 40 45  
 5 Glu Asp Ile Pro Phe Thr Arg Thr Asp Pro Val Val Ala Asp Tyr Lys  
 50 55 60  
 Tyr Asp Leu Lys Leu Gln Glu Tyr Gln Ser Ala Ile Lys Val Glu Pro  
 65 70 75 80  
 10 Ala Ser Pro Pro Tyr Tyr Ser Glu Lys Thr Gln Leu Tyr Asn Lys Pro  
 85 90 95  
 His Glu Glu Pro Ser Asn Ser Leu Met Ala Ile Glu Cys Arg Val Cys  
 100 105 110  
 Gly Asp Lys Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Glu Gly  
 115 120 125  
 15 Cys Lys Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys Leu Ile Tyr Asp  
 130 135 140  
 Arg Cys Asp Leu Asn Cys Arg Ile His Lys Lys Ser Arg Asn Lys Cys  
 145 150 155 160  
 20 Gln Tyr Cys Arg Phe Gln Lys Cys Leu Ala Val Gly Met Ser His Asn  
 165 170 175  
 Ala Ile Arg Phe Gly Arg Ile Ala Gln Ala Glu Lys Glu Lys Leu Leu  
 180 185 190  
 Ala Glu Ile Ser Ser Asp Ile Asp Gln Leu Asn Pro Glu Ser Ala Asp  
 195 200 205  
 25 Leu Arg Gln Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile Lys Ser  
 210 215 220  
 Phe Pro Leu Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly Lys Thr  
 225 230 235 240  
 30 Thr Asp Lys Ser Pro Phe Val Ile Tyr Asp Met Asn Ser Leu Met Met  
 245 250 255  
 Gly Glu Asp Lys Ile Lys Phe Lys His Ile Thr Pro Leu Gln Glu Gln  
 260 265 270  
 Ser Lys Glu Val Ala Ile Arg Ile Phe Gln Gly Cys Gln Phe Arg Ser  
 275 280 285  
 35 Val Glu Ala Val Gln Glu Ile Thr Glu Tyr Ala Lys Ser Ile Pro Gly  
 290 295 300  
 Phe Val Asn Leu Asp Leu Asn Asp Gln Val Thr Leu Leu Lys Tyr Gly  
 305 310 315 320  
 40 Val His Glu Ile Ile Tyr Thr Met Leu Ala Ser Leu Met Asn Lys Asp  
 325 330 335  
 Gly Val Leu Ile Ser Glu Gly Gln Gly Phe Met Thr Arg Glu Phe Leu  
 340 345 350  
 Lys Ser Leu Arg Lys Pro Phe Gly Asp Phe Met Glu Pro Lys Phe Glu  
 355 360 365

```

Phe Ala Val Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala
 370                               375                               380

Ile Phe Ile Ala Val Ile Ile Leu Ser Gly Asp Arg Pro Gly Leu Leu
385                               390                               395                               400

Asn Val Lys Pro Ile Glu Asp Ile Gln Asp Asn Leu Leu Gln Ala Leu
                               405                               410                               415

Glu Leu Gln Leu Lys Leu Asn His Pro Glu Ser Ser Gln Leu Phe Ala
                               420                               425                               430

Lys Leu Leu Gln Lys Met Thr Asp Leu Arg Gln Ile Val Thr Glu His
                               435                               440                               445

Val Gln Leu Leu Gln Val Ile Lys Lys Thr Glu Thr Asp Met Ser Leu
450                               455                               460

His Pro Leu Leu Gln Glu Ile Tyr Lys Asp Leu Tyr
465                               470                               475

```

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 41 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACTAGTGC GG CCGCCTAGGC CTCGATTTT TTTTTTTTTT T 41

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 16 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: Y
- (B) LOCATION: 3, 12 and 21
- (C) IDENTIFICATION METHOD: Y = C or T

(ix) FEATURE:

- (A) NAME/KEY: R
- (B) LOCATION: 6, and 15
- (C) IDENTIFICATION METHOD: R = A or G

(ix) FEATURE:

- (A) NAME/KEY: N
- (B) LOCATION: 9 and 18
- (C) IDENTIFICATION METHOD: N = G, A, C or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGYGARGGNT GYAARGGNTT YTT  
23

(2) INFORMATION FOR SEQ ID NO:5:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:  
Cys Glu Gly Cys Lys Gly Phe Phe  
1 5

(2) INFORMATION FOR SEQ ID NO:6:

- 15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:  
ACGTGACCTT TGTCTGGT 19

(2) INFORMATION FOR SEQ ID NO:7:

- 25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:  
30 TGACCT 6

(2) INFORMATION FOR SEQ ID NO:8:

- 35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:  
Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys  
40 1 5 10 15

WHAT IS CLAIMED IS:

1. An isolated and purified polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide.

5 2. The isolated and purified polynucleotide of Claim 1, wherein said polynucleotide is a DNA molecule.

3. The DNA molecule of Claim 2, wherein said encoded polypeptide is a peroxisome proliferator-activated receptor *gamma*.

4. The DNA molecule of Claim 2, wherein said encoded polypeptide comprises the amino acid residue sequence of SEQ ID NO:2.

10 5. The isolated and purified polynucleotide of Claim 1, wherein said polynucleotide comprises the nucleotide base sequence of SEQ ID NO:1.

15 6. An isolated and purified polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide, said polynucleotide preparable by a process comprising the steps of:

- 20
- (a) constructing a library of cDNA clones from a cell that expresses said polypeptide;
  - (b) screening the library with a radio-labelled oligonucleotide probe;
  - (c) identifying a clone that hybridizes to the probe; and
  - (d) isolating the hybridized clone from the library of unhybridized clones.



7. The isolated and purified polynucleotide of Claim 6, wherein said polynucleotide is prepared by a process comprising the steps of:

- (a) constructing a library of cDNA clones from a cell that expresses said polypeptide;
- (b) screening the library with a radio-labelled oligonucleotide probe;
- (c) identifying a clone that hybridizes to the probe; and
- (d) isolating the hybridized clone from the library of unhybridized clones.

8. An isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 1, wherein said polynucleotide hybridizes to a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide.

9. An isolated and purified peroxisome proliferator-activated receptor polypeptide.

10. The receptor polypeptide of Claim 9, wherein the polypeptide is a peroxisome proliferator-activated receptor *gamma* polypeptide.

11. The peroxisome proliferator-activated receptor polypeptide of Claim 9 that comprises the amino acid residue sequence of SEQ ID NO:2.

12. An expression vector comprising a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide.

13. The expression vector of Claim 12, wherein the polynucleotide comprises the nucleotide base sequence of SEQ ID NO:1.

14. A recombinant host cell transfected with a polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide.

5 15. The recombinant host cell of Claim 14, wherein the cell is transfected with the polynucleotide of SEQ ID NO:1.

16. A process of preparing a peroxisome proliferator-activated receptor polypeptide comprising:

- 10 (a) transfecting a cell with a polynucleotide that encodes the polypeptide to produce a transformed host cell; and  
(b) maintaining the transformed host cell under biological conditions sufficient for expression of the polypeptide.

17. The process of Claim 16 wherein said polynucleotide comprises the nucleotide base sequence of SEQ ID NO:1.

15 18. An antibody immunoreactive with a peroxisome proliferator-activated receptor polypeptide.

19. The antibody of Claim 18, wherein said antibody is a polyclonal or a monoclonal antibody.

20 20. A process of detecting a peroxisome proliferator-activated receptor polypeptide, wherein the process comprises:

- (a) immunoreacting the polypeptide with the antibody of Claim 18 to form an antibody-polypeptide conjugate; and  
(b) detecting the conjugate.

21. A process of detecting a messenger RNA transcript that encodes a peroxisome proliferator-activated receptor polypeptide, wherein the process comprises:

- 5 (a) hybridizing the messenger RNA transcript with a polynucleotide sequence that encodes the peroxisome proliferator-activated receptor polypeptide to form a duplex; and  
(b) detecting the duplex.

10 22. A pharmaceutical composition comprising a peroxisome proliferator-activated receptor polypeptide and a physiologically acceptable carrier.

23. A diagnostic assay kit for detecting the presence of a peroxisome proliferator-activated receptor polypeptide in a biological sample, said kit comprising a first container containing a first antibody capable of immunoreacting with said peroxisome proliferator-activated  
15 receptor polypeptide, wherein said first antibody is present in an amount sufficient to perform at least one assay.

24. A diagnostic assay kit for detecting the presence, in a biological sample, of a first polynucleotide that encodes a peroxisome proliferator-activated receptor polypeptide, said kit comprising a first  
20 container that contains a second polynucleotide identical or complementary to a segment of at least 10 contiguous nucleotide bases of SEQ ID NO: 1.

25. A process of screening a substance for its ability to interact with a peroxisome proliferator-activated receptor, said process comprising  
25 the steps of:



**ABSTRACT**

The invention relates generally to compositions of and methods for obtaining peroxisome proliferator-activated receptors. The invention relates as well to the DNA sequences encoding peroxisome proliferator-activated receptors, the recombinant vectors carrying those sequences, the recombinant host cells including either the sequences or vectors, and recombinant peroxisome proliferator-activated receptor polypeptides. By way of example, the invention discloses the cloning and functional expression of a peroxisome proliferator-activated receptor, designated PPAR- $\gamma$ , obtained from a human source. The invention includes as well, methods for using the isolated, recombinant peroxisome proliferator-activated receptor polypeptides in assays designed to select and improve substances capable of interacting with peroxisome proliferator-activated receptor polypeptides for use in diagnostic, drug design and therapeutic applications.

# AMINO ACID IDENTITY AMONG MEMBERS OF THE PPAR FAMILY

hPPAR $\gamma$	110	C	175	253	479
	A/B		D	T1	E
xPPAR $\gamma$	112	97%	70%	7%	91%
					477
xPPAR $\alpha$	108	84%	59%	30%	77%
					474
xPPAR $\beta$	30	74%	51%	24%	72%
					396
mPPAR $\alpha$	102	83%	57%	31%	82%
					468
hPPAR $\alpha$	102	83%	58%	35%	74%
					468
hNUC 1	72	83%	59%	26%	74%
					441
hRAR $\alpha$	87	65%	19.5%	4.7%	36%
					462

key: h= human m=murine x=xenopus

FIG. 1

DECLARATION

PATENT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

The below named inventors are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled "**Human Peroxisome Proliferator Activated Receptor Gamma: Compositions and Methods**" the specification of which:

\_\_\_\_\_ is attached hereto.

  ✓   was filed on October 8, 1993 as Application Serial No. 08/134,557

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

Priority Claimed

Yes/No

\_\_\_\_\_  
(Number)      (Country)      (Date Filed)

Yes/No

\_\_\_\_\_  
(Number)      (Country)      (Date Filed)

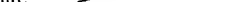
I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a), regarding events which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

\_\_\_\_\_  
(Application Serial No.)      (Filing Date)      (Status)

I hereby direct that all correspondence and telephone calls be addressed to **Thomas E. Northrup, Dressler, Goldsmith, Shore & Milnamow, Ltd., Two Prudential Plaza, Suite 4700, Chicago, IL 60601, (312) 616-5400.**

001030 07428500

[illegible]

Inventor's Signature: 

Residence: 2020 Lincoln Park West Apt 17-G  
(Include number, street name, city, state, and country)  
Chicago IL 60614

Post Office Address: N/A  
(If different from Residence Address)

Inventor's Signature: Sam Blumberg

Residence: 3435 Lebon Drive #1122  
(Include number, street name, city, state, and country)  
San Diego, CA 92122

Post Office Address: \_\_\_\_\_  
(If different from Residence Address)



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: )  
)  
Marianne E. Green & Bruce Blumberg )  
) Examiner: Unknown  
Serial No.: 08/134,557 )  
) Group Art Unit: Unknown  
Filed: October 8, 1993 )  
) Attorney Docket:  
For: Human Peroxisome Proliferator Activated )  
Receptor Gamma: Compositions and )  
Methods )

**POWER OF ATTORNEY**

Honorable Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

The undersigned, being Assignee of the entire interest in the above-identified application by virtue of an Assignment recorded in the United States Patent and Trademark Office as set forth below, hereby elects, under 37 C.F.R. §1.32, to prosecute the application to the exclusion of the inventors.

The Assignee hereby revokes any previous Powers of Attorney and appoints:

Max Dressler	Reg. No. 14,123	Joel E. Siegel	Reg. No. 25,440
Ernest Cheslow	Reg. No. 17,019	Lois P. Besanko	Reg. No. 27,855
Jack Shore	Reg. No. 17,551	Paul M. Odell	Reg. No. 28,332
John P. Milnamow	Reg. No. 20,635	Stephen D. Geimer	Reg. No. 28,846
Ronald L. Engel	Reg. No. 20,655	Paul M. Vargo	Reg. No. 29,116
Gerson E. Meyers	Reg. No. 21,160	Albert J. Brunett	Reg. No. 31,742
Allen J. Hoover	Reg. No. 24,103	Thomas E. Northrup	Reg. No. 33,268
Martin L. Katz	Reg. No. 25,011	Karl R. Fink	Reg. No. 34,161

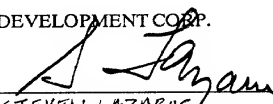
each members or associates of the law firm of DRESSLER, GOLDSMITH, SHORE & MILNAMOW, LTD., as its attorney so long as they remain with such law firm, with full power of substitution and revocation, to prosecute the application, to make alterations and amendments therein, to transact all business in the Patent and Trademark Office in connection therewith, and to receive the Letters Patent.

Please direct all communications as follows:

DRESSLER, GOLDSMITH, SHORE & MILNAMOW, LTD.  
Two Prudential Plaza, Suite 4700  
Chicago, IL 60601  
(312) 616-5400  
(312) 616-5460

ARCH DEVELOPMENT CORP.

Date: 3/21/94

By:   
Name: STEVEN LAZARUS  
Title: PRESIDENT & CEO